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Developmental Toxicity Potential of Nitroguanidine in Rats

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Division of Toxicology

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Developmental Toxicity Potential of Nitroguanidine in Rats (Toxicology Series 174)--
Coppes, Orner, and Korte

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S Beatrice 16 Feb 88

Edwin S. Beatrice (date)
COL, MC
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The potential of nitroguanidine to produce developmental toxicity was evaluated in pregnant Sprague-Dawley rats. Nitroguanidine, suspended in 1% carboxymethylcellulose, was administered at doses of 0, 100, 316, and 1000 mg/kg/day by oral gavage on Days 6 through 15 of gestation. Fetuses were delivered by cesarean section on Day 20, weighed, examined externally, and processed in either Bouin's solution for visceral examination or alizarin red stain for skeletal examination. Following a generalized failure to thrive, two animals in the 1000 mg/kg/day group died and one was terminated in a moribund condition. At necropsy, significant quantities of nitroguanidine were present in the stomachs of these three animals. Nitroguanidine given at 1000 mg/kg/day produced decreased food consumption, weight loss, dehydration, red urine, and red material on nose/whiskers in the dams during the treatment

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period and decreased weight gain from Day 0 to Day 20 of gestation. Fetuses from the 1000 mg/kg/day group were significantly smaller than controls with an increased incidence of retarded ossification of the sternebrae, caudal vertebrae, and pubis. There was no evidence of developmental toxicity of nitroguanidine in rats under conditions of this study. Nitroguanidine produced maternal and fetal toxicity at the 1000 mg/kg/day dose level. The no-observed-effect level was 316 mg/kg/day.

ABSTRACT

The potential of nitroguanidine to produce developmental toxicity was evaluated in pregnant Sprague-Dawley rats. Nitroguanidine, suspended in 1% carboxymethylcellulose, was administered at doses of 0, 100, 316, and 1000 mg/kg/day by oral gavage on Days 6 through 15 of gestation. Fetuses were delivered by cesarean section on Day 20, weighed, examined externally, and processed in either Bouin's solution for visceral examination or alizarin red stain for skeletal examination. Following a generalized failure to thrive, two animals in the 1000 mg/kg/day group died and one was terminated in a moribund condition. At necropsy, significant quantities of nitroguanidine were present in the stomachs of these three animals. Nitroguanidine given at 1000 mg/kg/day produced decreased food consumption, weight loss, dehydration, red urine, and red material on nose/whiskers in the dams during the treatment period and decreased weight gain from Day 0 to Day 20 of gestation. Fetuses from the 1000 mg/kg/day group were significantly smaller than controls with an increased incidence of retarded ossification of the sternebrae, caudal vertebrae, and pubis. There was no evidence of developmental toxicity of nitroguanidine in rats under conditions of this study. Nitroguanidine produced maternal and fetal toxicity at the 1000 mg/kg/day dose level. The no-observed-effect level was 316 mg/kg/day.

KEY WORDS: Developmental Toxicology, Teratology, Nitroguanidine, Rat

PREFACE

TYPE REPORT: Developmental Toxicity Study

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT: 3E16272JA835, Nitrocellulose-Nitroguanidine Projects; Work Unit 180; APC: TL09

GLP STUDY NUMBER: 85044

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ MSC

PRINCIPAL INVESTIGATOR: Valerie G. Coppes, BS

CO-PRINCIPAL INVESTIGATOR: Gayle A. Orner, BS, SP5

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, SOP's, and raw data will be retained in the LAIR Archives. Alizarin specimens will be retained in the LAIR Pathology Archives.

TEST SUBSTANCE: Nitroguanidine

INCLUSIVE STUDY DATES: 23 September 1985 - 18 March 1986

OBJECTIVE: The purpose of this study was to determine the developmental toxicity potential of nitroguanidine in pregnant Sprague-Dawley rats when administered orally during the period of organogenesis.

ACKNOWLEDGMENTS

Conrad R. Wheeler, PhD; SSG James D.Justus, SP4 Dean K. Magnuson, SP4 Theresa L. Polk, SP4 Scott L. Schwebe, SP4 James J. Fisher, and Richard Katona provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85044 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

9FEB88

DON W. KORTE, JR., PhD / DATE
MAJ, MSC
Study Director

9 Feb 88

VALERIE G. COPPERS, BS / DATE
DAC
Principal Investigator

9Feb88

GAYLE A. ORNER, BS / DATE
SP5
Co-Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

8 February 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for Study 85044

1. I hereby certify that the protocol was reviewed on 30 August 1985.

2. The report and raw data for this study were audited on 26 January 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
C, Quality Assurance

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Developmental Toxicity Potential of Nitroguanidine in Rats--Coppes et al

Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. The rat developmental toxicity study described in this report represented one of three studies (a rabbit developmental toxicity study and rat multigeneration reproductive study are the others) in the reproductive toxicity assessment being conducted as part of the health effects profile of nitroguanidine.

Objective of the Study

The purpose of this study was to determine the developmental toxicity potential of nitroguanidine in pregnant Sprague-Dawley rats when administered orally during the period of organogenesis.

MATERIALS

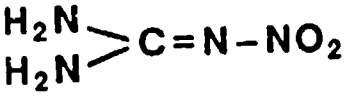
Test Substance

Chemical Name: Nitroguanidine

Chemical Abstract Service Registry No: 556-88-7

Toxicology Group Test Compound No.: TP036A

Molecular Structure



Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Lot No.: SOW84K010-A-001

Molecular Weight: 104.1

Physical State: White powder

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for nitroguanidine was a 1% solution of carboxymethylcellulose sodium salt, high viscosity (Sigma Chemical Co., St. Louis, MO). Nitroguanidine is not soluble in water at the dose levels tested. Carboxymethylcellulose holds nitroguanidine in a homogeneous suspension and is not developmentally toxic.

Animal Data

Young adult Sprague-Dawley rats were obtained from Bantin-Kingman, Fremont, CA. The study was conducted in two phases due to the number of animals required. Sixty-one female and 30 male Sprague Dawley rats were assigned to Phase I and 81 females and 40 males to Phase II. Two females from each phase were selected at random for quality control necropsy. Animals were identified by sequentially numbered metal eartags. Phase I animals were numbered 85D00907 through 85D00997, and Phase II animals 86D00001 through 86D00121. Additional animal data are presented in Appendix B.

A positive control study with ethylene thiourea established the Sprague-Dawley rat as a sensitive test system for developmental toxicity studies at LAIR (2).

Husbandry

Upon arrival at LAIR, rats were housed individually in wiremesh rack cages with automatic water dispensers for the quarantine period. Animals were fed Purina Certified Rodent Chow 5002 (Ralston Purina Company, St Louis, MO) and water (reverse osmosis Technic Central Systems, Series 300) ad libitum throughout the study. No contaminants or naturally occurring substances were expected to influence the study. During breeding, one male and two females were placed in polycarbonate cages with Alpha-dri bedding (Shepherd Specialty Papers, Kalamazoo, MI) and water bottles. After breeding, the males were returned to the wiremesh rack cages;

the pregnant females were housed individually in polycarbonate cages. The room temperature averaged 75.1 ± 2.0°F and the relative humidity averaged 45.0 ± 7.0% (mean ± S.D. of daily morning and evening hygrothermograph readings). The photoperiod was 12 hours of light per day.

METHODS

Methods used are described in detail in OP-STX-40 "Developmental Toxicity Study" (3) and were in accordance with Environmental Protection Agency Good Laboratory Practice Standards (4) and Health Effects Testing Guidelines (5).

Acclimation

Animals were acclimatized for three weeks prior to start of breeding. The females were acclimatized to brief periods of being handled each workday. Consequently, at the start of breeding, the females were docile and not resistant to being handled.

Group Assignment

Females were assigned to test groups by the weight-biased, stratified randomization method (OP-STX-78 "Stratified Randomization") on the Data General Eagle MV8000 computer which was based on the body weight at the start of breeding (6). Females were selected for quality control necropsy according to OP-ISG-21 "Animal Randomization Procedure" (7).

Dose Levels

Dose levels tested were 0, 100, 316, and 1000 mg/kg/day. Females were dosed daily between 0730 and 1200 hours from Day 6 through Day 15 of gestation by oral intubation using an 18-gauge, 3-inch gavage needle (Popper and Sons, Inc, New Hyde Park, NY). Dosing was conducted without sedation or anesthetization of the animals. The dose for each female was based on the Day 6 (Day 0 was the day sperm were detected in vagina) body weight and that dose was used throughout the treatment period. Phase I females were dosed from 21 Oct 85 through 9 Nov 85. Phase II females were dosed from 24 Feb 86 through 13 Mar 86.

Compound Preparation and Analysis

Initially, a smooth paste containing nitroguanidine and a small amount of vehicle was prepared in a mortar and pestle. Vehicle was then added gradually until the final volume was obtained. The concentrations prepared were 20 mg/ml for the 100 mg/kg/day dose, 63.2 mg/ml for the 316

mg/kg/day dose, and 200 mg/ml for the 1000 mg/kg/day dose. The dosing suspensions and vehicle control were given at a volume of 5 ml/kg body weight. The vehicle and dosing suspensions were prepared prior to the start of dosing for each study phase and refrigerated. Before the animals were dosed each day, the containers of dosing preparation were placed in a beaker of hot tap water for 15 to 30 minutes to bring the suspensions to room temperature. Chemical analyses for accuracy and homogeneity of the dosing suspensions are reported in Appendix C.

Breeding

After the quarantine period, each male was placed in a breeding cage with two females. Females were checked each morning for evidence of insemination. Day 0 for each female was the day sperm were observed in her vaginal smear. Sperm-positive females were separated from the male and caged individually. Those females which were not sperm positive at the completion of the breeding period were removed from the study.

Cesarean Section Procedure

Dams were weighed and euthanized with CO_2 gas on Day 20 of gestation. All females were examined, and non-pregnant ones were removed from the study. Gravid uteri were examined for number of implantation sites, resorptions, and live and dead fetuses. The fetuses, uterus, and ovaries were removed, the corpora lutea were counted, and the dam was examined for gross visceral signs of toxicity and reweighed. Each fetus was sexed, weighed, measured crown-to-rump, and examined externally. Fetuses were assigned alternately to either skeletal or visceral examination.

Fetuses assigned for skeletal examination were placed in 70% ethanol for several hours and eviscerated. They were then processed by the alizarin red S staining technique of Crary (8). After processing, the specimens were stored in glycerol with a few crystals of thymol to inhibit bacterial and mold growth. Fetuses assigned for visceral examination were placed in Bouin's solution. The body walls were pierced to allow penetration of the fixing solution.

Observations and Records

Pregnant females were weighed on Days 0, 6, 10, 15, and 20. Their feed also was weighed on Days 0, 6, 10, 15, and 20. Females were observed daily from Day 0 through Day 20 for clinical signs of toxicity, abortion, or premature delivery. Date, time, and amount of dosing suspension administered were recorded during the daily dosing on Days 6 through 15. At sacrifice, uterine data, gravid body weight,

number of corpora lutea, and results from gross examination of the dam were recorded. Dams were reweighed after the removal of the gravid uterus to determine the "Corrected Day 20" weight.

Fetal weight, crown-to-rump measurement, sex, and external examination findings from live fetuses were recorded. Fetuses processed in Bouin's solution were examined under a dissecting microscope by the modified Wilson freehand razor blade sectioning technique (9). The skeletons stained by alizarin were examined under low magnification on a light box for malformations, alignment, and degree of ossification. The ossified sternebrae, ribs, caudal vertebrae, metacarpals, and metatarsals were counted.

Schedule of Study Events

The study was divided into two phases to allow adequate time for animal care, fetal processing, and fetal examination. The historical listing of study events is given in Appendix D.

Statistical Analysis

The data were analyzed with BMDP software on a Data General Eagle MV8000 computer (10). Methods used are described by Hollander and Wolfe (11). Data from both phases were combined for analysis. In this report the term "significant" indicates a statistically significant difference. The litter or litter mean was used as the experimental unit. All tests were run at the .05 level of significance. The maternal body weights, weight changes, food consumption, and fetal weights and lengths were compared by one-way analysis of variance. Then, if a significant F occurred, the Student Newman-Keuls multiple range test was applied to the data. The implantation efficiency, percent resorptions, percent live and dead fetuses, and ossification data were compared by the one-way Kruskal-Wallis test. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used to determine which groups were different. The fetal examination findings were compared by chi-square analysis.

Changes/Deviations

The study was accomplished according to the protocol and addenda with no exceptions.

Raw Data and Final Report Storage

A copy of the final report, study protocol, addenda, raw data, SOPs, and an aliquot of test compound will be retained

in the LAIR Archives. Alizarin specimens will be retained in the LAIR Pathology Archives.

RESULTS

Maternal Data

The number of sperm-positive females in each group, number of animals that died during the study, and number of animals that were pregnant are presented in Table 1. Nitroguanidine did not affect the pregnancy rate.

Seven animals died during the study, and one moribund animal was terminated. Five of the seven animals died as a result of difficulties administering the concentrated dosing suspension. Animal 86D00006 in the 100 mg/kg/day group was found dead five hours after dosing on Day 14, 86D00062 in the 316 mg/kg/day group was found dead 40 minutes after dosing on Day 10, and three rats in the 1000 mg/kg/day group died (86D00059 and 86D00070 died immediately after dosing on Days 8 and 11, and 86D00040 was found dead on Day 17). At necropsy, these animals presented with uncollapsed lungs and dosing compound in the oropharyngeal cavity and lungs. Necropsy findings for two rats in the 1000 mg/kg/day group (85D00961 died on Day 16 and 85D00987 was euthanized in a moribund condition on Day 14) included a 1 cm in diameter soft dough-like ball of test compound in the stomach while findings from 85D00984, a 1000 mg/kg/day group animal that died on Day 14, included ingesta and a small amount of test compound in the stomach. Two of these rats that died, 86D00062 in the 316 mg/kg/day group and 86D00070 in the 1000 mg/kg/day group, were not pregnant. The clinical signs, body weights, and food consumption for these non-pregnant animals are not included in this report.

Individual maternal body weights and daily average food consumption of pregnant animals are presented in Appendix E and Appendix F, respectively. Results of maternal body weights, weight changes, and food consumption of pregnant females by group are in Table 2. When given at 1000 mg/kg/day, nitroguanidine produced weight loss during the treatment period, Days 6 to 15, and decreased weight gain during the study period, Day 0 to Corrected Day 20, in comparison to the control. Food consumption also was decreased significantly during the treatment period in the 1000 mg/kg/day dose group. Lower doses of nitroguanidine did not adversely affect maternal weight gain or food consumption.

Individual maternal clinical signs of pregnant animals are listed in Appendix G. Summaries of clinical signs by dose group during the pretreatment (Day 0 through Day 5),

treatment (Day 6 through Day 15),and posttreatment (Day 16 through Day 20) periods are found in Tables 3a,b, and c, respectively. Clinical signs, which occurred with a high frequency in the 1000 mg/kg/day group during the treatment period, included red urine, dehydration, red material on nose/whiskers, red material on forelimbs, and hunched posture. Clinical signs occurred in 100% of the 1000 mg/kg/day group versus 39% of the control group during the treatment period and in 29% of the 1000 mg/kg/day group animals compared with 9% in the control group during the posttreatment period.

Cesarean/Fetal Data

The individual uterine data are listed in Appendix H, the mean uterine data by group in Table 4. Nitroguanidine had no effect on the number of corpora lutea, implantations, resorptions, and live and dead fetuses.

The individual number of live males and females per litter, and the average fetal weight and length per litter are given in Appendix I; the group means are in Table 5. Nitroguanidine did not affect the male-to-female ratio. Male and female fetuses from the 1000 mg/kg/day dose group were significantly lighter in weight and shorter in length than the controls. There was no size difference in the 100 and 316 mg/kg/day dose group fetuses in comparison to the controls.

Individual external examination findings are presented in Appendix J. A summary by dose group is in Table 6. A variation seen at low frequency was hematoma. Three fetuses had external malformations. One control group fetus had anasarca and abnormal body shape in which the body was short and thick, particularly through the neck. In the 316 mg/kg/day group, one fetus had bilateral anophthalmia, hypoplastic pinnae, absent lower jaw, and abnormal body shape (square) and one fetus from a different litter had anasarca. These variations and malformations are not dose-related.

Individual visceral examination findings are in Appendix K. A summary by dose group is presented in Table 7. Slightly dilated renal pelvis was the most frequently observed visceral variation, occurring in all groups with the highest incidence in the control group. Dilated 4th ventricle of the brain was also present in all groups: three fetuses in one litter in the control, one fetus in one litter in the 100 mg/kg/day group, two fetuses in two litters in the 316 mg/kg/day group, and six fetuses in five litters in the 1000 mg/kg/day group. Visceral malformations occurred in three fetuses. The control group fetus with anasarca and abnormal body shape at external examination also had marked enlargement of the adrenals. The fetus in the 316 mg/kg/day

group with anasarca at external examination also had multiple visceral malformations which included partial cleft palate, abnormalities of the heart ventricles, and hypoplasia of the lungs. One fetus from another litter in the 316 mg/kg/day group had a small lens in the left eye (approximately 1/4 normal size), and the left eye was in a more medial position than normal. These spontaneous malformations are not dose-related.

Skeletal variations and malformations are described in Appendix L, a group summary in Table 8. Two fetuses had skeletal malformations. One fetus in the 316 mg/kg/day group had multiple malformations of the head. The external examination on this fetus revealed anophthalmia and absent lower jaw. The skeletal examination findings were cleft palate and small, slit-like orbit with straight zygomatic arch. The mandible was present, but extremely short and fused on the midline. One fetus in the 1000 mg/kg/day dose group had a malformed orbit in which the frontal bone ended abruptly in an arch over the orbit; it did not curve inward to form the eyesocket.

The mean numbers of ossified sternebrae, caudal vertebrae, metacarpals, and metatarsals/fetus/litter are in Appendix M; the summary by group is found in Table 9. The fetuses from the 1000 mg/kg/day group had significantly fewer ossified sternebrae and caudal vertebrae than the controls. The 1000 mg/kg/day group fetuses also had fewer ossified metacarpals and metatarsals, but this decrease was not significant. Additionally, the fetuses from the 1000 mg/kg/day group had a higher incidence of reduced ossification of the pubis than the controls. There was a dose-related decrease in the incidence of rudimentary lumbar ribs, ranging from 17.7% in the control to 11.8% in the 1000 mg/kg/day dose group.

The individual incidence of external, visceral, and skeletal variations and malformations is found in Appendix N, and the individual incidence of any variation and malformation is found in Appendix O. A summary by dose group of the effect of nitroguanidine on the incidence of fetal malformations and variations is presented in Table 10. There was no significant difference in the rate of malformations among the dose groups. The number of fetuses, but not the number of litters, with skeletal variations was increased in the 1000 mg/kg/day dose group in comparison to the control.

Table 1

Effect of Nitroguanidine on Survival and Pregnancy

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Sperm-positive females	27	27	23	27
Females that died	0	1	1	6
Nongravid	0	0	1	1
Gravid	0	1	0	5
Females examined on Day 20	27	26	22	21
Nongravid	4	6	4	2
Gravid	23	20	18	19
Resorptions only	0	1	0	1
With live fetuses	23	19	18	18
Females that were gravid	23	21	18	24

Table 2

Effect of Nitroguanidine on Maternal Body Weights and Average Daily Food Consumption[a]

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Average body weight				
Day 0	270 ± 22	265 ± 28	279 ± 25	270 ± 21
Day 6	298 ± 27	298 ± 30	311 ± 30	303 ± 24
Day 10	310 ± 25	308 ± 32	319 ± 30	275 ± 29[b]
Day 15	334 ± 27	325 ± 41	346 ± 27	282 ± 37[b]
Day 20 Corrected	320 ± 21	307 ± 40	329 ± 24	293 ± 22[b]
Weight Change				
Days Corrected 20 - 0	49 ± 13	41 ± 16	50 ± 16	25 ± 15[b]
Days 15 - 6	36 ± 12	27 ± 15	35 ± 15	-20 ± 39[b]
Average daily food consumption				
Days 0 - 6	23 ± 3	24 ± 2	25 ± 4	24 ± 3
Days 6 - 15	24 ± 2	23 ± 3	24 ± 2	13 ± 5[b]
Days 15 - 20	26 ± 3	24 ± 4	27 ± 2	26 ± 4

[a] Mean ± S.D. in g for pregnant females.
[b] Significantly different from control by Student Newman-Keuls multiple range test, $p < 0.05$.

Table 3a

Maternal Clinical Signs[a] - Pretreatment (Days 0-5)

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Number of animals observed	23	21	18	24
Number with signs	2	0	1	0
Red material on nose			1	
Dehydrated - water not available	2		1	

[a]Pregnant females.

Table 3b

Maternal Clinical Signs[a] - Treatment (Days 6-15)

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Number of animals observed	23	21	18	24
Number with signs	9	14	8	24
Red urine	1			20
White pasty material in urine				1
Red material on nose/whiskers	1	3	1	14
Red material on forelimbs				7
Ear bleeding at eartag site			1	
Red mucous vaginal discharge				1
Red material on ears				2
Rough hair coat				1
Alopecia forelimb		3		1
Alopecia hindlimb		1		
Alopecia ear, irritation from eartag	1	1		
Dehydrated	1			16
Dehydrated - water not available	1	1	1	
Soft stools		2	1	
Diarrhea			1	3
Small feces				1
Irritable				3
Hyperactive				1
Ataxia				1
Inactive				2
Increased startle reflex		1		2
Convulsions				1
Twitching			1	
Tense, jittery				3
Hunched posture				6
Stiff, short steps				2
Cyanosis				2
Tremors				2
Eyes squinting	1			2
Eyes weeping				1

[a]Pregnant females.

Table 3b (Continued)

Maternal Clinical Signs[a] - Treatment (Days 6-15)

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Sound production; growling	1	2		
Increased rate of respiration	1			
Increased depth of respiration				1
Dried compound in throat from previous dosing				2
During dosing procedure:				
Blood in nose/mouth/dosing needle	3	3		1
Small amount of compound in mouth	3	6	5	4
Moderate amount of compound in mouth	1	3	1	1
Large amount of compound out of mouth			1	1
Death or euthanized in moribund condition		1		3
Necropsy results:				
1 cm ball of compound in stomach				1
Stomach, small intestines distended with gas				1
Stomach contained ingesta, small amount of compound				1
Mineralization of kidneys				1
Pyelonephritis				1
Dosing compound in oral cavity and lungs		1		1

[a]Pregnant females.

Table 3c

Maternal Clinical Signs[a] - Posttreatment (Days 16 - 20)

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Number of animals observed	23	20	18	21
Number with signs	2	7	1	6
Red urine				1
Red material on nose		1	1	3
Red material/stain ears		1		1
Red material forelimbs				1
Rough hair coat				1
Alopecia forelimbs		3		1
Alopecia hindlimbs		2		
Alopecia chest		1		
Alopecia ear, irritation from eartag		1		
No feces in cage				1
Inactive	1	1		
Hunched posture				1
Cyanosis				1
Eyes squinting	1			
Sunken eyes				1
Increased rate of respiration				1
Decreased depth of respiration				1
Death				2
At Cesarean section:				
Amniotic fluid brownish-yellow				1
Embryo sac distended with excess fluid				1
Necropsy results:				
1 cm ball of compound in stomach				1
Dosing compound in oral cavity and lungs				1

[a]Pregnant females.

Table 4

Effect of Nitroguanidine on Mean Uterine Data[a]

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Corpora lutea	17.8 ± 3.8	17.0 ± 3.1	17.5 ± 2.9	18.0 ± 3.9
Implantations	13.4 ± 3.1	13.2 ± 3.4	13.4 ± 2.6	12.8 ± 3.9
Implantation efficiency[b]	77.6 ± 20.0	77.9 ± 21.0	77.2 ± 13.0	74.9 ± 26.0
Resorptions	1.1 ± 1.2	1.0 ± 1.5	0.9 ± 1.3	1.7 ± 2.9
Percent resorptions[c]	8.3 ± 9.1	11.3 ± 23.0	6.2 ± 8.2	12.9 ± 24.0
Number of fetuses				
Live	12.3 ± 3.3	12.3 ± 3.6	12.4 ± 2.2	11.2 ± 4.6
Percent live[d]	99.6 ± 1.9	100.0	99.5 ± 2.0	100.0
Dead	0.04 ± 0.2	0.0	0.1 ± 0.2	0.0
Percent dead[e]	0.4 ± 1.9	0.0	0.5 ± 2.0	0.0

[a] Mean ± S.D./litter
[b] Implantations/corpora lutea x 100
[c] Resorptions/implantations x 100
[d] Live/(live + dead) x 100
[e] Dead/(live + dead) x 100

Table 5

Effect of Nitroguanidine on Mean Litter Size, Sex, Weight, and Length[a]

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Number of fetuses	12.3 ± 3.3	12.9 ± 2.2	12.4 ± 2.2	11.8 ± 3.8
Number of males	6.1 ± 1.9	6.1 ± 2.2	5.7 ± 2.1	6.1 ± 2.6
Number of females	6.2 ± 2.7	6.8 ± 2.0	6.7 ± 2.9	5.7 ± 2.5
Percent males	50.6 ± 13.0	46.5 ± 14.0	47.4 ± 20.0	52.4 ± 13.0
Weight (g) males	3.6 ± 0.4	3.8 ± 0.7	3.7 ± 0.6	3.1 ± 0.4[b]
Weight (g) females	3.4 ± 0.4	3.5 ± 0.7	3.5 ± 0.5	2.9 ± 0.4[b]
Length (cm) males	3.6 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	3.5 ± 0.2[b]
Length (cm) females	3.6 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.4 ± 0.2[b]

[a] Mean ± S.D./litter
[b] Significantly different from control by Student Newman-Keuls multiple range test, $p < 0.05$.

Table 6

Effect of Nitroguanidine on
Fetal External Malformations and Variations

	Nitroguanidine (mg/kg/day)			
Examination Finding	0	100	316	1000
Fetuses/Litters	282/23	245/19	224/18	212/18
Malformations				
Anasarca	1/1		1/1	
Abnormal body shape	1/1		1/1	
Anophthalmia			1/1	
Hypoplastic pinnae			1/1	
Lower jaw absent			1/1	
Variations				
Hematoma	1/1	1/1		1/1
Lips scalloped at edge				1/1

A single fetus may have more than one abnormality and, therefore, would occur more than once in this table.

Table 7

Effect of Nitroguanidine on
Fetal Visceral Malformations and Variations

Examination Finding	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Fetuses/Litters	135/23	118/19	107/18	102/18
Malformations				
Enlarged adrenals	1/1			
Small lens			1/1	
Eyeball medial position			1/1	
Cleft palate			1/1	
Abnormal heart			1/1	
Lobular lung surface			1/1	
Hypoplastic lungs			1/1	
Variations				
Dilated 4th ventricle	3/1	1/1	2/2	6/5
Dilated lateral ventricle				1/1
Dilated nasal cavity				1/1
Coarse textured, discolored lung			1/1	
Mottled coloration of liver				1/1
Dilated renal pelvis	11/5	5/3	3/3	3/2
Undescended testes			1/1	

A single fetus may have more than one abnormality and, therefore, would occur more than once in this table.

Table 8

Effect of Nitroguanidine on
Fetal Skeletal Malformations and Variations

	Nitroguanidine (mg/kg/day)			
Examination Finding	0	100	316	1000
Fetuses/Litters	147/23	127/19	117/18	110/18
Malformations				
Abnormal orbit			1/1	1/1
Malformed mandible			1/1	
Cleft palate			1/1	
Extra vertebrae			1/1	

A single fetus may have more than one abnormality and, therefore, would occur more than once in this table.

Table 8 (Concluded)

Effect of Nitroguanidine on
Fetal Skeletal Malformations and Variations

Examination Finding	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Variations				
Skull: retarded ossification	9/4	1/1	2/2	6/4
Supraoccipital misshaped			1/1	
Small orbit	1/1			
Zygomatic arch:				
retarded ossification	1/1			
Straight mandible				1/1
Vertebral arch:				
retarded ossification	1/1			1/1
projections		1/1	1/1	
Vertebral centra:				
retarded ossification	2/2		1/1	2/2
abnormal shape			1/1	
not ossified				1/1
Sternebrae:				
split	1/1			8/3
fewer than 3 ossified	3/3	3/3		10/6
abnormal shape			1/1	
Ribs:				
rudimentary lumbar	26/11	21/13	14/9	13/9
rudimentary 2nd		1/1	1/1	
lumbar ribs (fully formed)				2/1
bunched, not parallel			1/1	
Caudal vertebrae:				
fewer than 3 ossified	8/5	4/4		28/9
Pubis:				
short		1/1	2/1	4/2
retarded ossification	3/3	4/3	6/3	23/9
not ossified	2/2	2/2	1/1	6/2
Slightly curved femur			1/1	
Metatarsals:				
fewer than 4 ossified	4/3	2/2	1/1	14/6

A single fetus may have more than one abnormality and, therefore, would occur more than once in this table.

Table 9

Effect of Nitroguanidine on Mean Fetal Ossification Data[a]

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Sternebrae	4.7 ± 0.8	5.2 ± 0.8	5.1 ± 0.8	3.8 ± 1.0[b]
Caudal vertebrae	4.3 ± 0.8	4.5 ± 0.8	4.7 ± 0.8	3.4 ± 0.8[b]
Metacarpals/paw	3.2 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	3.0 ± 0.0
Metatarsals/paw	4.0 ± 0.0	4.1 ± 0.2	4.0 ± 0.0	3.9 ± 0.3

[a] Mean + S.D./litter.
[b] Significantly different from control by Mann-Whitney test, $p < 0.05$.

Table 10

Effect of Nitroguanidine on the Incidence of Fetal Malformations and Variations

	Nitroguanidine (mg/kg/day)			
	0	100	316	1000
Number fetuses/litters	282/23	245/19	224/18	212/18
Any (External/Visceral/Skeletal)				
Malformations	1/1	0/0	3/3	1/1
Variations	49/18	35/18	32/15	67/14
External examination				
Malformations	1/1	0/0	2/2	0/0
Variations	1/1	1/1	0/0	2/2
Visceral examination				
Number fetuses/litters	135/23	118/19	107/18	102/18
Malformations	1/1	0/0	2/2	0/0
Variations	12/5	6/4	6/6	11/8
Skeletal examination				
Number fetuses/litters	147/23	127/19	117/18	110/18
Malformations	0/0	0/0	1/1	1/1
Variations	37/16	28/16	26/13	55/14

DISCUSSION

The health effects of nitroguanidine are being determined because of the Army's decision to incorporate nitroguanidine in its triple-base propellants. Previously, this laboratory showed that nitroguanidine was slightly toxic in rats and mice following acute oral administration, was nonirritating to the skin and eyes of rabbits, and was nonreactive in a dermal sensitization study in guinea pigs (12). A subchronic toxicity study in rats with doses as high as a "limit dose" of 1000 mg/kg/day mixed in the diet for 14 days produced no definitive toxicological effects (13). This lack of toxicity was supported by metabolic fate studies that indicated that nitroguanidine was 100% absorbed following oral administration and was excreted unchanged in the urine, 60-80% within the first 8 hours (13)

The predominant sign of maternal toxicity observed in this study was death in three animals (two animals died and one moribund animal was terminated) in the 1000 mg/kg/day group. It is doubtful that these deaths were attributable to a direct pharmacological effect for two reasons. First, nitroguanidine when administered in the feed at similar dose levels in a 14-day study was well tolerated (13). Second, balls of dough-like compound were present in the stomachs of two animals, and in the third animal, there was also evidence at necropsy of test compound in the stomach. The general failure to thrive of these animals suggested that the high concentrations of nitroguanidine necessary to administer the 1000 mg/kg/day dose by oral intubation interfered with the digestive processes of the animals in this group. This is supported by the other signs of maternal toxicity observed in the 1000 mg/kg/day animals. These signs included decreased food consumption, weight loss during the treatment period, decreased weight gain during the gestation period, and an increased incidence of clinical signs. The deaths of the five animals with uncollapsed lungs and dosing compound in the oropharyngeal cavity and lungs were attributed to difficulties administering the concentrated dosing suspension. There were no adverse maternal effects in the 100 or 316 mg/kg/day groups.

The four primary manifestations of developmental toxicity are death of the conceptus, malformation, retarded development, and functional deficit. This study was designed to screen for the first three. In a developmental toxicity test the fetal examination findings may range in severity from slightly retarded development or minor variations to major malformations. Retarded development may be transitory, for example, caused by decreased maternal food consumption, and the retarded offspring may catch up quickly after birth or after weaning. Minor variations from normal may not have an adverse effect on the function and quality of life of the offspring. Major structural malformations, such as malformed

or missing organs or limbs, can either be life threatening or severely limit the functioning and longevity of the offspring. A test substance is considered developmentally toxic if, when administered at a dose level which is not overtly maternally toxic, it produces malformations at a significantly higher incidence than in the controls. Although variations are not as serious as malformations, a significantly increased incidence of variations, in comparison to the controls, is a sign of some fetal or maternal toxicity (14). Spontaneous malformations are those that occur randomly, usually at low frequency, and are of unknown cause, and whose incidence is not dose-related.

In this study each fetus was examined externally at cesarean section and then for either visceral or skeletal abnormalities. The findings on each fetus were described and categorized as either variations or malformations, depending on the severity or whether the changes were permanent. Those findings categorized as variations included such transitory findings as retarded ossification (includes those fetuses with fewer than three sternebrae, fewer than three metacarpals, and fewer than four metatarsals ossified), dilated brain ventricles, dilated renal pelvis, undescended testes, hematoma, and minor deviations from normal that may or may not be permanent such as slightly misshapen bones, small eye orbit, discoloration or coarse texture of organs. Findings of more serious consequence that were categorized as malformations included cleft palate, malformed mandible, extra vertebrae, anasarca, anophthalmia, abnormal heart, marked enlargement of the adrenals, small lens, and hypoplastic lungs.

The retarded development of the fetuses in the 1000 mg/kg/day group resulted in an increased number of skeletal variations in comparison to the controls. These fetuses were significantly lighter in weight, shorter in length, with fewer ossified sternebrae and caudal vertebrae. This retarded development could be attributed to maternal toxicity rather than to a direct effect of nitroguanidine on the fetus. The 1000 mg/kg/day group dams lost weight, consumed less food during the treatment period, and gained less weight during the entire gestation period.

The malformations observed in this study are considered spontaneous because they are not dose-related and occurred at a low frequency. Five fetuses (one in the control group, three in the 316 mg/kg/day group and one in the 1000 mg/kg/day group) out of a total of 963 fetuses in the study were malformed. This incidence is similar to that published by Palmer (15) and historical incidence from this laboratory (2).

CONCLUSION

There was no evidence of developmental toxicity of nitroguanidine in rats under conditions of this study. Nitroguanidine produced maternal and fetal toxicity at the 1000 mg/kg/day dose level. The no-observed-effect level was 316 mg/kg/day.

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Appendix A Chemical Data
Appendix B Animal Data
Appendix C Chemical Analysis
Appendix D Schedule of Study Events
Appendix E Individual Maternal Body Weights
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APPENDICES

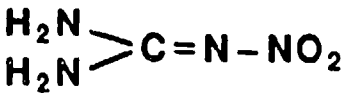
CHEMICAL DATA

Chemical name: Nitroguanidine (NGu)

Other listed names: Guanidine, Nitro; alpha-Nitroguanidine; beta-Nitroguanidine

LAIR Code: TP036A

Structural formula:



Molecular formula: $CH_4N_4O_2$

Molecular weight: 104.1

pH range of dosing suspensions: 6.7 - 7.4[1]

Physical state: White Powder

Melting point: 232° C[2]

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Lot No. SOW84K010-A-001

Analytical data/purity:
The major peaks in the infrared spectrum of the compound were observed at 3450, 3396, 3342, 3278, 3201, 1666, 1634, 1525, 1404, 1314, 1151, 1045, 782 cm^{-1}.[3] The spectrum obtained for the test compound in our lab was identical to the Sadtler standard spectrum for nitroguanidine[4]. HPLC showed only one peak (retention time 4.9 min)[5]. The conditions employed were as follows: column, Brownlee RP-18 (4.6 x 250 mm); solvent, 10% methanol-90% water; flow rate, 0.7 ml/min; oven temperature, 50°C; monitoring wavelength, 265nm.

Stability:
Stable in 1% carboxymethylcellulose for at least nine months (see Appendix C-2).

1. Wheeler CR. Nitrocellulose-Nitroguanidine Projects Laboratory Notebook #85-12-022, p. 26. Letterman Army Institute of Research, Presidio of San Francisco, CA.
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5. Wheeler, CR. Nitrocellulose-Nitroguanidine Projects Laboratory Notebook #85-12-022, pp. 24-25. Letterman Army Institute of Research, Presidio of San Francisco, CA.

ANIMAL DATA

Species: Rattus norvegicus

Strain: Sprague-Dawley

Source: Bantin-Kingman
Fremont, California

Condition of animals at start of study: Normal

	Phase I	Phase II
Date of Birth:		
Males	24 Jul 85	12 Nov 85
Females	24 Jun 85	14 Nov 85
Age (days) at start of breeding:		
Males	82	97
Females	112	95
Weight (g) range at start of breeding:		
Males	312 - 444	389 - 468
Females	259 - 332	209 - 290
Number of animals:		
Males	30	40
Females	61	81

HOMOGENEITY[a]

A suspension of nitroguanidine (200 mg/ml, 300 ml) was prepared in 1% carboxymethylcellulose. This suspension was subsequently used to prepare two more dilute suspensions of approximately 60 mg/ml (20 ml) and 20 mg/ml (20 ml) in 20-ml vials. The suspensions were stirred well, and aliquots of 1 ml were removed from the top, middle, and bottom layers of each suspension. The aliquots were transferred to either 500- or 1000-ml volumetric flasks and diluted to volume with water. After one more dilution (see table below) the optical absorbance at 264 nm was determined.

The concentration of the original suspension was then calculated using the dilution and absorbance data. A comparison of the individual values to the mean value of the appropriate group showed no deviation larger than 3%.

Target Concentration mg/ml	Area Sample	1st Dilution ml	2nd Dilution ml	Absorbance at 264 nm	Concentration mg/ml
20	top	500	5	1.305	23.4
	middle			1.304	23.4
	bottom			1.302	23.4
60	top	1000	10	1.021	73.4
	middle			1.043	75.0
	bottom			1.076	77.3
200	top	1000	25	1.150	206.6
	middle			1.163	209.0
	bottom			1.135	203.9

[a]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-12-022, p. 27-29. Letterman Army Institute of Research. Presidio of San Francisco, CA.

CHEMICAL ANALYSIS OF DOSING SUSPENSIONS[a]

All dosing suspensions were analyzed by transferring 1- or 5- ml aliquots of suspension to a volumetric flask and diluting to volume. An aliquot of the first dilution was subsequently transferred to a second volumetric flask and diluted to volume (for total dilution see dilution factor in table below). The absorbance spectrum (200-340 nm) of the final dilution was determined with a UV/VIS spectrophotometer. The absorbance at 264 nm was then used to calculate the concentration of nitroguanidine according to the following equation which is based on Beer's law:

$$\text{Concentration (Conc.)} = \frac{\text{Absorbance} \times \text{dilution factor} \times \text{molecular weight of nitroguanidine 104 g/mole}}{\text{molar extinction coefficient (14,470)}}$$

Date Prepared	Date Analyzed	Target Conc. mg/ml	Dilution Factor	Absorbance nm	Conc. Determined by Analysis mg/ml	% Target Conc.
18 Oct 85	2 Jul 86	20.0	2,500	1.104	19.8	99
18 Oct 85	11 Jul 86	63.2	10,000	0.900	64.7	102
18 Oct 85	11 Jul 86	200.0	20,000	1.463	210.3	105
21 Feb 86	2 Jul 86	20.0	2,500	1.004	18.0	90
21 Feb 86	11 Jul 86	63.2	10,000	0.855	61.5	97
21 Feb 86	11 Jul 86	200.0	20,000	1.397	200.8	100

All concentrations of nitroguanidine were within 10% of the target concentration. In each case, the pattern of the spectrum obtained on scanning from 200 to 340 corresponded exactly to that expected for nitroguanidine. The long interval of time between the date of preparation and analysis shows that suspensions of nitroguanidine in 1% carboxymethylcellulose are stable for at least nine months.

[a]Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023.2, pp. 11-16. Letterman Army Institute of Research, Presidio of San Francisco, CA.

SCHEDULE OF STUDY EVENTS

DATE	EVENT
3 Sep 85	Date protocol approved.
23 Sep 85	Rats for Phase I arrived at LAIR.
14 - 26 Oct 85	Phase I breeding.
21 Oct - 9 Nov 85	Phase I females dosed.
4 - 14 Nov 85	Cesarean sections, Phase I females.
28 Jan 86	Rats for Phase II arrived at LAIR.
17 - 28 Feb 86	Phase II breeding.
24 Feb - 13 Mar 86	Phase II females dosed.
10 - 18 March 86	Cesarean sections, Phase II females.

Individual Maternal Body Weights[a]
Control Animals

Maternal ID	Day of Gestation						Weight Change	
					Gravid	Correct		
	0	6	10	15	20	20	20C-0[b]	15-6[c]
85D00939	288	310	321	355	397	337	49	45
85D00944	273	307	316	345	387	336	63	38
85D00945	264	300	310	331	383	325	61	31
85D00955	285	318	315	349	416	323	38	31
85D00958	302	338	353	382	438	346	44	44
85D00959	275	308	325	344	398	320	45	36
85D00974	306	335	348	377	452	351	45	42
85D00976	294	314	333	351	402	320	26	37
85D00986	289	344	353	373	434	368	79	29
85D00988	316	337	355	383	436	347	31	46
86D00001	250	271	289	317	359	319	69	46
86D00005	231	272	283	294	366	286	55	22
86D00010	258	234	277	309	368	317	59	75
86D00014	267	308	308	328	378	326	59	20
86D00016	247	278	294	305	354	301	54	27
86D00025	244	278	296	317	386	303	59	39
86D00039	265	279	279	310	382	303	38	31
86D00052	269	276	285	312	385	306	37	36
86D00055	254	278	299	298	361	282	28	20
86D00071	275	306	309	342	405	331	56	36
86D00076	254	295	295	314	345	297	43	19
86D00077	249	290	290	329	381	304	55	39
86D00079	266	286	301	328	398	309	43	42

[a] Weights in g.
[b] Study period (Day 20 Corrected - Day 0).
[c] Treatment period (Day 15 - Day 6).

Individual Maternal Body Weights[a]
100 mg/kg/day Nitroguanidine Animals

Maternal ID	Day of Gestation						Weight Change	
	0	6	10	15	Gravid 20	Correct 20	20C-0[b]	15-6[c]
85D00947	270	298	310	286	386	308	38	-12
85D00950	280	333	341	370	432	348	68	37
85D00953	291	338	353	380	423	359	68	42
85D00956	338	369	368	401	481	388	50	32
85D00972	316	351	377	390	455	380	64	39
85D00991	283	300	317	341	402	312	29	41
85D00992	300	330	340	381	429	340	40	51
86D00006	253	294	287	[d]				
86D00020	238	274	281	284	358	275	37	10
86D00024	248	273	295	300	354	297	49	27
86D00029	259	294	302	317	338	290	31	23
86D00031	262	294	289	308	375	280	18	14
86D00032	255	286	301	316	372	305	50	30
86D00037	237	260	275	294	341	255	18	34
86D00043	268	305	318	[e]	386	316	48	[e]
86D00046	254	287	293	326	381	309	55	39
86D00050	214	247	251	248	230	229	15	1
86D00051	245	276	284	296	354	289	44	20
86D00054	256	287	295	316	360	285	29	29
86D00073	245	279	293	312	382	290	45	33
86D00081	255	291	298	309	369	288	33	18

[a] Weights in g.
[b] Study period (Day 20 Corrected - Day 0).
[c] Treatment period (Day 15 - Day 6).
[d] Animal died on Day 14.
[e] Missing data.

Individual Maternal Body Weights[a]
316 mg/kg/day Nitroguanidine Animals

Maternal ID	Day of Gestation						Weight Change	
					Gravid	Correct		
	0	6	10	15	20	20	20C-0[b]	15-6[c]
85D00914	332	354	363	392	452	362	30	38
85D00915	268	313	306	335	384	317	49	22
85D00917	290	334	349	379	447	352	62	45
85D00938	288	331	344	367	434	361	73	36
85D00954	273	309	321	334	391	318	45	25
85D00968	276	311	333	372	440	343	67	61
85D00977	319	352	360	381	438	371	52	29
85D00980	277	314	322	351	399	327	50	37
85D00985	311	322	335	353	400	319	8	31
85D00993	307	350	357	377	438	355	48	27
86D00008	259	295	308	338	405	333	74	43
86D00022	244	229	291	309	372	306	62	80
86D00026	261	288	300	317	379	290	29	29
86D00027	251	296	289	308	364	302	51	12
86D00058	252	293	295	316	343	306	54	23
86D00064	279	314	326	351	406	342	63	37
86D00068	275	300	247	332	385	316	41	32
86D00080	256	286	298	317	367	301	45	31

[a] Weights in g.
[b] Study period (Day 20 Corrected - Day 0).
[c] Treatment period (Day 15 - Day 6).

Individual Maternal Body Weights[a]
1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Day of Gestation						Weight Change	
	0	6	10	15	Gravid 20	Correct 20	20C-0[b]	15-6[c]
85D00942	285	312	294	319	383	322	37	7
85D00948	300	350	302	284	345	311	11	-66
85D00951	265	319	339	356	415	326	61	37
85D00952	271	309	293	310	381	300	29	1
85D00961	292	333	257	209	[d]			-124
85D00971	300	327	307	304	382	310	10	-23
85D00981	274	305	288	307	363	298	24	2
85D00982	294	332	300	322	368	312	18	-10
85D00984	278	298	261	[e]				
85D00987	306	345	283	[e]				
85D00995	299	329	292	279	346	299	0	-50
86D00002	266	286	263	284	366	291	25	- 2
86D00003	257	289	297	314	383	303	46	25
86D00004	257	282	264	269	344	277	20	-13
86D00015	273	307	318	298	360	317	44	- 9
86D00034	244	279	248	270	328	264	20	- 9
86D00035	245	265	248	282	345	272	27	17
86D00036	257	284	242	264	341	283	26	-20
86D00040	266	301	252	207	[f]			-94
86D00041	223	266	227	231	270	247	24	-35
86D00048	255	275	235	264	342	269	14	-11
86D00059	253	286	[g]					
86D00067	269	297	240	247	286	276	7	-50
86D00075	258	295	277	294	327	284	26	- 1

[a] Weights in g.
[b] Study period (Day 20 Corrected - Day 0).
[c] Treatment period (Day 15 - Day 6).
[d] Animal died on Day 16.
[e] Animal died on Day 14.
[f] Animal died on Day 17.
[g] Animal died on Day 8.

Individual Maternal Food Consumption[a]
Control Animals

Maternal ID	Days of Gestation				
	0-6	6-10	10-15	6-15	15-20
85D00939	23.2	22.5	26.8	24.9	27.4
85D00944	24.3	25.0	25.6	25.3	24.4
85D00945	23.3	24.5	23.4	23.9	24.4
85D00955	24.8	19.0	21.2	20.2	23.8
85D00958	26.7	26.8	26.4	26.6	27.2
85D00959	25.8	28.0	23.0	25.2	25.2
85D00974	26.2	30.3	24.0	26.8	27.0
85D00976	24.3	25.0	21.6	23.1	25.6
85D00986	28.0	26.3	24.8	25.5	32.2
85D00988	26.2	27.3	24.8	25.9	29.6
86D00001	21.0	24.8	24.8	24.8	28.6
86D00005	25.0	23.8	21.0	22.2	24.6
86D00010	16.0	16.0	25.4	21.2	26.6
86D00014	23.5	23.3	22.8	23.0	24.4
86D00016	24.7	19.8	21.8	20.9	24.2
86D00025	19.2	22.0	21.4	21.7	24.0
86D00039	22.7	24.0	22.4	23.1	27.6
86D00052	13.0	26.5	24.2	25.2	25.4
86D00055	20.8	23.0	21.2	22.0	18.8
86D00071	26.0	25.3	26.0	25.7	26.8
86D00076	24.7	22.5	23.4	23.0	24.4
86D00077	23.7	24.3	25.4	24.9	25.6
86D00079	21.0	25.0	24.8	24.9	28.2

[a]Average daily food consumption in g.

Individual Maternal Food Consumption[a]
100 mg/kg/day Nitroguanidine Animals

Maternal ID	Days of Gestation				
	0-6	6-10	10-15	6-15	15-20
85D00947	20.7	21.5	18.6	19.9	21.8
85D00950	27.8	26.8	26.8	26.8	25.6
85D00953	27.5	26.8	27.6	27.2	28.6
85D00956	27.2	22.3	23.4	22.9	30.2
85D00972	26.3	29.5	27.2	28.2	28.4
85D00991	22.2	28.5	30.4	29.6	19.8
85D00992	26.3	27.0	26.6	26.8	28.6
86D00006	23.3	19.5	[b]		
86D00020	23.5	21.3	15.8	18.2	26.0
86D00024	23.8	24.0	17.0	20.1	26.0
86D00029	23.8	21.3	20.4	20.8	16.6
86D00031	22.5	14.8	22.2	18.9	23.2
86D00032	23.2	23.5	23.4	23.4	24.6
86D00037	20.3	21.0	19.2	20.0	20.8
86D00043	24.7	26.0	24.6	25.2	24.8
86D00046	20.0	20.0	23.6	22.0	25.6
86D00050	19.8	20.0	16.0	17.8	15.6
86D00051	24.3	22.0	21.0	21.4	26.6
86D00054	22.8	24.3	22.6	23.4	21.8
86D00073	24.5	24.5	22.4	23.3	27.2
86D00081	24.7	22.3	23.6	23.0	26.2

[a]Average daily food consumption in g.
[b]Animal died on Day 14.

Individual Maternal Food Consumption[a]
316 mg/kg/day Nitroguanidine Animals

Maternal ID	Days of Gestation				
	0-6	6-10	10-15	6-15	15-20
85D00914	28.0	26.0	27.4	26.8	29.4
85D00915	24.2	19.3	21.4	20.5	27.2
85D00917	30.3	27.0	29.0	28.1	29.4
85D00938	28.2	27.5	26.8	27.1	29.6
85D00954	25.8	24.0	21.2	22.4	25.2
85D00968	17.8	26.3	28.4	27.5	29.6
85D00977	26.2	24.8	23.8	24.2	28.6
85D00980	26.5	24.3	24.4	24.4	26.6
85D00985	24.0	23.0	23.2	23.1	27.6
85D00993	29.0	25.0	26.0	25.6	26.6
86D00008	23.2	22.8	25.0	24.0	26.8
86D00022	16.3	24.8	23.0	23.8	26.2
86D00026	25.8	30.8	16.4	22.8	23.8
86D00027	24.0	19.8	20.2	20.0	24.0
86D00058	23.8	22.8	25.0	24.0	25.0
86D00064	27.5	27.0	26.2	26.6	31.6
86D00068	23.3	24.5	22.0	23.1	24.2
86D00080	22.7	24.0	22.0	22.9	24.2

[a]Average daily food consumption in g.

Individual Maternal Food Consumption[a]
1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Days of Gestation				
	0-6	6-10	10-15	6-15	15-20
85D00942	25.5	14.8	18.4	16.8	27.6
85D00948	28.8	12.5	9.2	10.7	22.6
85D00951	25.8	23.0	22.6	22.8	27.4
85D00952	21.0	16.0	13.4	14.6	24.6
85D00961	27.3	1.8	1.4	1.6	[b]
85D00971	27.3	15.0	11.6	13.1	24.4
85D00981	24.5	18.0	18.6	18.3	27.0
85D00982	26.2	10.3	14.4	12.6	26.0
85D00984	24.0	7.5	[c]		
85D00987	28.3	6.3	[c]		
85D00995	27.2	11.0	8.0	9.3	20.6
86D00002	19.7	16.3	20.2	18.5	30.6
86D00003	25.7	22.0	22.8	22.4	39.8
86D00004	23.7	13.3	10.2	11.6	23.0
86D00015	24.7	14.8	16.6	15.8	30.6
86D00034	22.7	9.3	14.4	12.1	24.0
86D00035	22.2	10.8	18.0	14.8	27.2
86D00036	22.2	6.0	10.2	8.3	27.0
86D00040	25.8	0.0	8.6	4.8	[d]
86D00041	21.7	9.0	10.6	9.9	20.6
86D00048	19.3	9.5	13.8	11.9	26.6
86D00059	21.7	[e]			
86D00067	22.0	6.5	10.0	8.4	22.0
86D00075	23.8	18.3	17.8	18.0	25.2

[a] Average daily food consumption in g.
[b] Animal died on Day 16.
[c] Animal died on Day 14.
[d] Animal died on Day 17.
[e] Animal died on Day 8.

Individual Maternal Clinical Signs - Control Animals

Maternal ID	Study Day(s)	Date(s)	Signs
86D00001	16	8 Mar 86	Inactive
86D00005	6	25 Feb 86	Small amount of compound in mouth after dosing
	9	28 Feb 86	Blood on nose during dosing procedure
	14	5 Mar 86	Small amount of compound in mouth after dosing
86D00010	6	24 Feb 86	Dehydrated, water not available
	7	25 Feb 86	Increased rate of respiration
	8 - 10	26 - 28 Feb 86	Dehydrated
86D00025	7	25 Feb 86	Red material on nose
86D00039	2	24 Feb 86	Dehydrated, water not available
	14	8 Mar 86	Red urine; alopecia left ear, irritation from eartag
86D00052	5	24 Feb 86	Dehydrated, water not available
	13	4 Mar 86	Small amount of compound in mouth after dosing
86D00055	14	4 Mar 86	Blood on dosing needle after dosing; sound production, growling
86D00076	11 - 19	1 - 9 Mar 86	Squinting, left eye
	14	4 Mar 86	Moderate amount of compound in mouth after dosing
86D00077	14	4 Mar 86	Small amount of compound in mouth after dosing
86D00079	9	28 Feb 86	Blood on nose during dosing procedure

Individual Maternal Clinical Signs - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
85D00347	14	29 Oct 85	Increased startle reflex
	15	30 Oct 85	Dehydrated, water not available
86D00006	6	24 Feb 86	Small amount of compound in mouth after dosing
	8	26 Feb 86	Small amount of compound in mouth after dosing
	14	4 Mar 86	Found dead approximately 5 hours after dosing; necropsy report - dosing material in oral cavity and lungs
86D00020	12	4 Mar 86	Small amount of blood in mouth during dosing
	12 - 13	4 - 5 Mar 86	Red material on nose
	14	6 Mar 86	Small amount of blood in mouth during dosing
	16	8 Mar 86	Red stain right ear
86D00024	11 - 19	3 - 11 Mar 86	Alopecia forelimbs
	13	5 Mar 86	Moderate amount of compound in mouth after dosing
	18 - 19	10 - 11 Mar 86	Alopecia hindlimbs
86D00029	15	7 Mar 86	Sound production, growling
	18	10 Mar 86	Red material on nose
86D00031	6	24 Feb 86	Small amount of blood in mouth after dosing; small amount of compound in mouth after dosing; sound production, growling
	13	3 Mar 86	Soft stool
	13 - 19	3 - 9 Mar 86	Alopecia forelimbs
	16 - 19	6 - 9 Mar 86	Alopecia chest

Individual Maternal Clinical Signs - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
86D00037	12	4 Mar 86	Small amount of blood in mouth after dosing; small amount of compound in mouth after dosing
	18	10 Mar 86	Inactive
86D00043	14 - 15	4 - 5 Mar 86	Small amount of compound in mouth after dosing
86D00046	12	2 Mar 86	Soft stool
86D00050	9	27 Feb 86	Small amount of compound in mouth after dosing
	14	4 Mar 86	Red material on nose
	15	5 Mar 86	Moderate amount of compound in mouth after dosing
86D00051	13 - 19	6 - 12 Mar 86	Alopecia forelimbs
	15 - 19	8 - 12 Mar 86	Alopecia hindlimbs
86D00054	12 - 18	2 - 8 Mar 86	Alopecia left ear, left ear infected at eartag site
86D00073	8	28 Feb 86	Red material on nose
	13	5 Mar 86	Moderate amount of compound in mouth after dosing
86D00081	10	28 Feb 86	Small amount of compound in mouth after dosing

Individual Maternal Clinical Signs - 316 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
85D00954	11	29 Oct 85	Small amount of blood in mouth after dosing
85D00968	1	19 Oct 85	Red material on nose
	4	22 Oct 85	Dehydrated, water not available
85D00980	8	23 Oct 85	Soft stools
	13 - 14	28 - 29 Oct 85	Large amount of compound out of mouth after dosing
86D00008	15	5 Mar 86	Small amount of compound in mouth after dosing
86D00022	6	24 Feb 86	Dehydrated, water not available
86D00026	8	28 Feb 86	Red material on nose
	10	2 Mar 86	Red material on whiskers
	12	4 Mar 86	Small amount of compound in mouth after dosing
	13	5 Mar 86	Moderate amount of compound in mouth after dosing
	16	8 Mar 86	Red material on nose
86D00027	9	27 Feb 86	Diarrhea
86D00058	6 - 7	24 - 25 Feb 86	Small amount of compound in mouth after dosing
	10	28 Feb 86	Ear bleeding at eartag site
	12	2 Mar 86	Twitching
86D00068	14	5 Mar 86	Small amount of compound in mouth after dosing

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
85D00942	8	24 Oct 85	Red urine; increased depth of respiration
	9	25 Oct 85	Red material on nose
85D00948	10 - 15	27 Oct - 1 Nov 85	Dehydrated
	15	1 Nov 85	Red material on nose
85D00951	12	29 Oct 85	Irritable; tense, jittery
85D00952	10	27 Oct 85	Red urine; dehydrated
	11	28 Oct 85	Red urine; irritable
85D00961	7 - 9	24 - 26 Oct 85	Red urine
	7	24 Oct 85	Red material on nose
	8 - 15	25 Oct - 1 Nov 85	Dehydrated
	10	27 Oct 85	Dried compound in throat from previous dosing; red material on forelimbs
	11 - 15	28 Oct - 1 Nov 85	Red material on nose
	12	29 Oct 85	Red urine
	13	30 Oct 85	Large amount of compound out of mouth during dosing
	13 - 15	30 Oct - 1 Nov 85	Red material on forelimbs
	15	1 Nov 85	Hunched posture; stiff, short steps; cyanosis; large amount of compound out of mouth during dosing
	16	2 Nov 85	Found dead in cage; necropsy report - stomach contained 1 cm in diameter soft round ball of compound

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
85D00971	9 - 10	2 - 3 Nov 85	Red urine
	12	5 Nov 85	Dried compound in throat from previous dosing
	13	6 Nov 85	Red mucous vaginal discharge
85D00981	9	24 Oct 85	Red urine
85D00982	7	25 Oct 85	Red urine
	10	28 Oct 85	Dehydrated; red material on forelimbs
	13	31 Oct 85	Hunched posture; tense, jittery
	20	7 Nov 85	At cesarean section, amniotic fluid brownish-yellow
85D00984	7	25 Oct 85	Red urine; dehydrated
	9 - 11	27 - 29 Oct 85	Red material on nose
	10 - 11	28 - 29 Oct 85	Dehydrated; red material on forelimbs
	12	30 Oct 85	Dehydrated; small feces
	13	31 Oct 85	Dehydrated; hunched posture; inactive; red material on nose; eyes squinting; eyes weeping; stiff short steps; tremors
	14	1 Nov 85	Found dead in cage; necropsy report - stomach contained ingesta and small amount of compound

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
85D00987	7	22 Oct 85	Red urine
	9 - 10	24 - 25 Oct 85	Dehydrated
	11 - 14	26 - 29 Oct 85	Red urine; dehydrated; red material on nose
	12	27 Oct 85	Hyperactive
	13	28 Oct 85	Red material on forelimbs; ataxia
	13 - 14	28 - 29 Oct 85	Hunched posture; tense, jittery; eyes squinting
	14	29 Oct 85	Inactive; tremors; increased startle reflex; convulsions; euthanized in moribund condition; necropsy report - stomach contained 1 x 1.5 cm soft ball of compound; stomach and intestines distended with gas; mineralization of kidneys; pyelonephritis
85D00995	10	26 Oct 85	Dehydrated
	14	30 Oct 85	Red material on nose; red material on forelimbs
	14 - 15	30 - 31 Oct 85	Dehydrated
86D00002	8	27 Feb 86	Red urine
	10 - 13	1 - 4 Mar 86	Dehydrated
	12	3 Mar 86	Red urine
	14	5 Mar 86	Small amount of compound in mouth after dosing
86D00003	11	5 Mar 86	Moderate amount of compound in mouth after dosing
	13 - 14	7 - 8 Mar 86	Blood in mouth after dosing
	14	8 Mar 86	Red material on nose
	14 - 19	8 - 13 Mar 86	Alopecia forelimbs

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
86D00004	6	26 Feb 86	Red material on nose
	7	27 Feb 86	Red urine
	8	28 Feb 86	Diarrhea; red material on nose
	11 - 15	3 - 7 Mar 86	Red material on nose
	15 - 16	7 - 8 Mar 86	Red material on ears
86D00015	7	27 Feb 86	Red material on nose
	9	1 Mar 86	Red urine
	10	2 Mar 86	Red material on nose
	11 - 15	3 - 7 Mar 86	Red urine
	16 - 17	8 - 9 Mar 86	Red material on nose
86D00034	7	25 Feb 86	Red urine; red material on nose
	8	26 Feb 86	Red material on nose
	11 - 14	1 - 4 Mar 86	Dehydrated
	13	3 Mar 86	Red material on nose
	14 - 15	4 - 5 Mar 86	Red urine
86D00035	7 - 11	25 Feb - 5 Mar 86	Red urine
	10 - 13	28 Feb - 3 Mar 86	Dehydrated
86D00036	7	25 Feb 86	Red material on nose
	7 - 11	25 Feb - 1 Mar 86	Red urine
	10 - 15	28 Feb - 5 Mar 86	Dehydrated
	11 - 16	1 - 6 Mar 86	Red material on nose
	15	5 Mar 86	Small amount of compound in mouth after dosing
	20	10 Mar 86	At cesarean section, 1 embryo sac distended to 3 times normal size with excess fluid

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
86D00040	7	27 Feb 86	Red urine
	8 - 16	28 Feb - 8 Mar 86	Dehydrated
	11	3 Mar 86	Red urine; increased startle reflex
	13	5 Mar 86	Red urine
	14	6 Mar 86	Red material on nose
	15	7 Mar 86	Diarrhea
	15 - 16	7 - 8 Mar 86	Hunched posture; red urine; red material on nose;red material on forelimbs; rough hair coat; cyanosis
	16	8 Mar 86	Sunken eyes; no feces in cage; Increased rate of respiration; decreased depth of respiration
	17	9 Mar 86	Found dead in cage; necropsy report - dosing compound in oral cavity and lungs
86D00041	8	26 Feb 86	Diarrhea; red urine
	9 - 14	27 Feb - 4 Mar 86	Dehydrated
	15	5 Mar 86	Small amount of compound in mouth after dosing
86D00048	7	27 Feb 86	Red urine
	8 - 11	28 Feb - 3 Mar 86	Dehydrated
86D00059	7 - 8	25 - 26 Mar 86	Red urine
	8	26 Mar 86	Died immediately after dosing; necropsy report - dosing compound in oral cavity and lungs

Individual Maternal Clinical Signs - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Study Day(s)	Date(s)	Signs
86D00067	7	5 Mar 86	White pasty material in urine
	8	6 Mar 86	Red urine
	8 - 11	6 - 9 Mar 86	Red material right ear
	9	7 Mar 86	Dehydrated
	9 - 13	7 - 11 Mar 86	Red material on nose
	10	8 Mar 86	Irritable
	10 - 14	8 - 12 Mar 86	Red material forelimbs
	11 - 12	9 - 10 Mar 86	Hunched posture
	12	10 Mar 86	Red urine
	12 - 14	10 - 12 Mar 86	Dehydrated
86D00075	7	25 Feb 86	Red material on nose
	10	28 Feb 86	Red urine; dehydrated; small amount of compound in mouth after dosing
	12	2 Mar 86	Red material on nose
	13	3 Mar 86	Red urine
	15	5 Mar 86	Small amount of compound in mouth after dosing

Individual Uterine Data - Control Animals

Maternal ID	Corpora Lutea	Implant	% [a] Implant	Resorptions	% [b] Resorptions	Number of Fetuses: Dead	%Dead[c]	Live	%Live[d]
85D00939	17	9	53	0	0	0	0	9	100
85D00944	13	10	77	2	20	0	0	8	100
85D00945	17	13	76	4	31	0	0	9	100
85D00955	18	17	94	0	0	0	0	17	100
85D00958	18	18	100	0	0	0	0	18	100
85D00959	15	13	87	0	0	0	0	13	100
85D00974	18	18	100	1	6	0	0	17	100
85D00976	25	14	56	1	7	0	0	13	100
85D00986	16	15	94	4	27	1	9	10	91
85D00988	24	16	67	2	13	0	0	14	100
86D00001	27	7	26	1	14	0	0	6	100
86D00005	17	15	88	0	0	0	0	15	100
86D00010	16	12	75	1	8	0	0	11	100
86D00014	17	14	82	2	14	0	0	12	100
86D00016	16	11	69	2	18	0	0	9	100
86D00025	17	15	88	2	13	0	0	13	100
86D00039	23	12	52	0	0	0	0	12	100
86D00052	15	15	100	1	7	0	0	14	100
86D00055	17	16	94	1	6	0	0	15	100
86D00071	14	12	86	0	0	0	0	12	100
86D00076	15	7	47	0	0	0	0	7	100
86D00077	13	13	100	0	0	0	0	13	100
86D00079	22	16	73	1	6	0	0	15	100

[a] Implantations/corpora lutea x 100
[b] Resorptions/implantations x 100
[c] Dead/(live + dead) x 100
[d] Live/(live + dead) x 100

Individual Uterine Data - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	Corpora Lutea	Implant	% Implant[a]	Resorptions	% Resorptions[b]	Number of Fetuses			
						Dead	%Dead[c]	Live	%Live[d]
85D00947	15	14	93	0	0	0	0	14	100
85D00950	22	16	73	1	6	0	0	15	100
85D00953	24	10	42	0	0	0	0	10	100
85D00956	23	17	74	0	0	0	0	17	100
85D00972	16	12	75	0	0	0	0	12	100
85D00991	17	16	94	0	0	0	0	16	100
85D00992	17	16	94	1	6	0	0	15	100
86D00020	14	13	93	1	8	0	0	12	100
86D00024	17	15	88	5	33	0	0	10	100
86D00029	15	14	93	5	36	0	0	9	100
86D00031	16	15	94	1	7	0	0	14	100
86D00032	17	12	71	0	0	0	0	12	100
86D00037	19	12	63	1	8	0	0	11	100
86D00043	16	13	81	0	0	0	0	13	100
86D00046	15	12	80	0	0	0	0	12	100
86D00050	11	1	9	1	100	0		0	
86D00051	15	13	87	2	15	0	0	11	100
86D00054	15	13	87	0	0	0	0	13	100
86D00073	18	16	89	1	6	0	0	15	100
86D00081	18	14	78	0	0	0	0	14	100

[a] Implantations/corpora lutea x 100
[b] Resorptions/implantations x 100
[c] Dead/(live + dead) x 100
[d] Live/(live + dead) x 100

Individual Uterine Data - 316 mg/kg/day Nitroguanidine Animals

Maternal ID	Corpora Lutea	Implant	% [a] Implant	Resorptions	% [b] Resorptions	Number of Fetuses			
						Dead	%Dead[c]	Live	%Live[d]
85D00914	17	16	94	1	6	0	0	15	100
85D00915	15	13	87	2	15	0	0	11	100
85D00917	22	16	73	1	6	0	0	15	100
85D00939	17	14	82	1	7	0	0	13	100
85D00958	15	12	80	0	0	0	0	12	100
85D00968	21	15	71	0	0	0	0	15	100
85D00977	18	15	83	4	27	0	0	11	100
85D00980	19	12	63	0	0	0	0	12	100
85D00985	17	15	88	1	7	0	0	14	100
85D00993	21	17	81	3	18	0	0	14	100
86D00008	14	13	93	1	8	1	8	11	92
86D00022	14	11	79	0	0	0	0	11	100
86D00026	21	15	71	0	0	0	0	15	100
86D00027	16	16	100	3	19	0	0	13	100
86D00058	13	6	46	0	0	0	0	6	100
86D00064	15	11	73	0	0	0	0	11	100
86D00068	20	13	65	0	0	0	0	13	100
86D00080	20	12	60	0	0	0	0	12	100

[a] Implantations/corpora lutea x 100
[b] Resorptions/implantations x 100
[c] Dead/(live + dead) x 100
[d] Live/(live + dead) x 100

Individual Uterine Data - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Corpora Lutea	Implant	% [a] Implant	Resorptions	% [b] Resorptions	Number of Fetuses: Dead	%Dead[c]	Live	%Lived[d]
85D00942	20	17	85	2	12	0	0	15	100
85D00948	22	9	41	3	33	0	0	6	100
85D00951	16	16	100	1	6	0	0	15	100
85D00952	16	14	88	0	0	0	0	14	100
85D00971	16	15	94	0	0	0	0	15	100
85D00981	12	11	92	1	9	0	0	10	100
85D00982	25	17	68	6	35	0	0	11	100
85D00995	15	14	93	2	14	0	0	12	100
86D00002	17	16	94	0	0	0	0	16	100
86D00003	26	16	62	0	0	0	0	16	100
86D00004	15	15	100	1	7	0	0	14	100
86D00015	22	7	32	0	0	0	0	7	100
86D00034	16	14	88	0	0	0	0	14	100
86D00035	15	14	93	2	14	0	0	12	100
86D00036	12	12	100	1	8	0	0	11	100
86D00041	21	3	14	0	0	0	0	3	100
86D00048	19	15	79	1	7	0	0	14	100
86D00067	19	12	63	12	100	0		0	
86D00075	18	7	39	0	0	0	0	7	100

[a] Implantations/corpora lutea x 100
[b] Resorptions/implantations x 100
[c] Dead/(live + dead) x 100
[d] Live/(live + dead) x 100

Fetal Sex, Weight, and Length - Control Animals

Maternal ID	No.	Sex			Mean Weight(g) ± S.D.		Mean Length(cm) ± S.D.	
		M	F	M %	Males	Females	Males	Females
85D00939	9	6	3	67	3.4 ± 0.2	3.6 ± 0.3	3.7 ± 0.1	3.7 ± 0.1
85D00944	8	4	4	50	3.8 ± 0.2	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
85D00945	9	6	3	67	3.8 ± 0.3	3.4 ± 0.3	3.7 ± 0.1	3.6 ± 0.2
85D00955	17	7	10	41	3.6 ± 0.3	3.3 ± 0.2	3.7 ± 0.2	3.6 ± 0.1
85D00958	18	5	13	28	3.1 ± 0.1	2.9 ± 0.3	3.5 ± 0.1	3.3 ± 0.1
85D00959	13	7	6	54	4.0 ± 0.3	3.8 ± 0.2	3.8 ± 0.1	3.8 ± 0.1
85D00974	17	10	7	59	3.7 ± 0.3	3.6 ± 0.2	3.8 ± 0.1	3.7 ± 0.1
85D00976	13	5	8	38	3.8 ± 0.1	3.7 ± 0.2	3.8 ± 0.0	3.8 ± 0.1
85D00986	10	5	5	50	3.3 ± 0.4	3.1 ± 0.4	3.5 ± 0.2	3.4 ± 0.2
85D00988	14	6	8	43	4.1 ± 0.3	3.9 ± 0.2	3.8 ± 0.1	3.7 ± 0.1
86D00001	6	2	4	33	3.4 ± 0.1	3.0 ± 0.3	3.5 ± 0.1	3.3 ± 0.3
86D00005	15	6	9	40	3.3 ± 0.1	3.2 ± 0.2	3.4 ± 0.1	3.4 ± 0.1
86D00010	11	6	5	55	3.2 ± 0.1	2.8 ± 0.3	3.5 ± 0.1	3.4 ± 0.3
86D00014	12	6	6	50	3.3 ± 0.2	2.9 ± 0.2	3.5 ± 0.1	3.5 ± 0.2
86D00016	9	6	3	67	3.3 ± 0.2	3.1 ± 0.4	3.5 ± 0.1	3.4 ± 0.2
86D00025	13	9	4	69	4.8 ± 0.2	4.7 ± 0.2	4.1 ± 0.1	3.9 ± 0.1
86D00039	12	8	4	67	3.8 ± 0.5	3.7 ± 0.1	3.6 ± 0.2	3.6 ± 0.1
86D00052	14	5	9	36	3.5 ± 0.2	3.2 ± 0.2	3.6 ± 0.1	3.4 ± 0.1
86D00055	15	7	8	47	3.2 ± 0.3	2.9 ± 0.4	3.5 ± 0.1	3.5 ± 0.2
86D00071	12	6	6	50	3.6 ± 0.3	3.4 ± 0.2	3.5 ± 0.1	3.4 ± 0.1
86D00076	7	4	3	57	4.2 ± 0.1	3.6 ± 0.2	3.7 ± 0.2	3.6 ± 0.1
86D00077	13	4	9	31	3.9 ± 0.1	3.4 ± 0.5	3.7 ± 0.1	3.6 ± 0.2
86D00079	15	10	5	67	3.5 ± 0.3	3.5 ± 0.2	3.6 ± 0.1	3.6 ± 0.1

Fetal Sex, Weight, and Length - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	No.	Sex			Mean Weight(g) ± S.D.		Mean Length(cm) ± S.D.	
		M	F	M %	Males	Females	Males	Females
85D00947	14	7	7	50	3.7 ± 0.2	3.5 ± 0.3	3.7 ± 0.2	3.7 ± 0.1
85D00950	15	5	10	33	3.2 ± 0.5	3.2 ± 0.2	3.6 ± 0.2	3.5 ± 0.1
85D00953	10	6	4	60	3.4 ± 0.1	2.8 ± 0.6	3.8 ± 0.1	3.5 ± 0.4
85D00956	17	8	9	47	3.3 ± 0.2	2.7 ± 0.2	3.7 ± 0.1	3.4 ± 0.1
85D00972	12	7	5	58	3.2 ± 0.2	3.1 ± 0.2	3.6 ± 0.2	3.6 ± 0.1
85D00991	16	11	5	69	3.4 ± 0.3	3.2 ± 0.2	3.7 ± 0.1	3.5 ± 0.1
85D00992	15	5	10	33	3.9 ± 0.2	3.8 ± 0.2	3.9 ± 0.1	3.7 ± 0.1
86D00020	12	4	8	33	5.1 ± 0.2	4.6 ± 0.8	4.1 ± 0.1	3.9 ± 0.3
86D00024	10	5	5	50	3.5 ± 0.1	3.3 ± 0.3	3.6 ± 0.1	3.5 ± 0.2
86D00029	9	2	7	22	3.4 ± 0.0	2.9 ± 0.4	3.5 ± 0.0	3.3 ± 0.2
86D00031	14	10	4	71	5.1 ± 0.3	4.8 ± 0.4	4.1 ± 0.2	3.9 ± 0.1
86D00032	12	4	8	33	3.8 ± 0.1	3.5 ± 0.2	3.6 ± 0.1	3.6 ± 0.1
86D00037	11	5	6	45	5.7 ± 0.2	5.2 ± 0.2	4.1 ± 0.1	4.0 ± 0.0
86D00043	13	7	6	54	3.0 ± 0.3	3.0 ± 0.2	3.5 ± 0.2	3.5 ± 0.1
86D00046	12	7	5	58	3.6 ± 0.2	3.4 ± 0.1	3.7 ± 0.0	3.5 ± 0.1
86D00051	11	4	7	36	3.3 ± 0.1	3.1 ± 0.3	3.4 ± 0.1	3.3 ± 0.2
86D00054	13	5	8	38	4.0 ± 0.2	3.7 ± 0.2	3.8 ± 0.1	3.6 ± 0.3
86D00073	15	5	10	33	3.8 ± 0.4	3.6 ± 0.2	3.6 ± 0.2	3.5 ± 0.1
86D00081	14	8	6	57	3.8 ± 0.2	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1

Fetal Sex, Weight, and Length - 316 mg/kg/day Nitroguanidine Animals

Maternal ID	No.	Sex			Mean Weight(g) ± S.D.		Mean Length(cm) ± S.D.	
		M	F	M %	Males	Females	Males	Females
85D00914	15	3	12	20	3.6 ± 0.3	3.4 ± 0.3	3.8 ± 0.1	3.6 ± 0.1
85D00915	11	4	7	36	3.8 ± 0.1	3.5 ± 0.2	3.8 ± 0.0	3.6 ± 0.1
85D00917	15	9	6	60	3.7 ± 0.1	3.5 ± 0.3	3.7 ± 0.1	3.7 ± 0.2
85D00938	13	5	8	38	3.2 ± 0.2	2.9 ± 0.3	3.6 ± 0.1	3.5 ± 0.2
85D00954	12	9	3	75	3.5 ± 0.3	3.3 ± 0.3	3.7 ± 0.1	3.5 ± 0.2
85D00968	15	4	11	27	3.4 ± 0.3	3.5 ± 0.3	3.6 ± 0.2	3.6 ± 0.1
85D00977	11	1	10	9	3.9	3.5 ± 0.2	3.8	3.7 ± 0.1
85D00980	12	7	5	58	4.0 ± 0.3	3.8 ± 0.2	3.9 ± 0.1	3.8 ± 0.1
85D00985	14	7	7	50	3.4 ± 0.3	3.3 ± 0.2	3.7 ± 0.2	3.5 ± 0.1
85D00993	14	6	8	43	3.6 ± 0.1	3.4 ± 0.2	3.7 ± 0.1	3.6 ± 0.1
86D00008	11	9	2	82	5.4 ± 0.5	4.6 ± 0.6	4.2 ± 0.2	4.0 ± 0.1
86D00022	11	4	7	36	5.1 ± 0.4	4.8 ± 0.3	4.2 ± 0.2	4.1 ± 0.1
86D00026	15	6	9	40	3.7 ± 0.2	3.4 ± 0.3	3.5 ± 0.1	3.4 ± 0.1
86D00027	13	6	7	46	3.3 ± 0.2	3.1 ± 0.2	3.5 ± 0.1	3.4 ± 0.1
86D00058	6	5	1	83	3.8 ± 0.1	3.0	3.5 ± 0.1	3.4
86D00064	11	5	6	45	3.3 ± 0.6	3.4 ± 0.2	3.4 ± 0.3	3.4 ± 0.1
86D00068	13	7	6	54	3.0 ± 0.4	3.1 ± 0.2	3.3 ± 0.3	3.4 ± 0.1
86D00080	12	6	6	50	3.9 ± 0.2	3.6 ± 0.1	3.8 ± 0.1	3.6 ± 0.2

Fetal Sex, Weight, and Length - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	No.	Sex M	Sex F	Sex M %	Mean Weight (g) ± S.D. Males	Mean Weight (g) ± S.D. Females	Mean Length (cm) ± S.D. Males	Mean Length (cm) ± S.D. Females
85D00942	15	5	10	33	2.7 ± 0.2	2.7 ± 0.4	3.3 ± 0.1	3.3 ± 0.2
85D00948	6	4	2	67	3.2 ± 0.3	2.5 ± 0.3	3.4 ± 0.1	3.1 ± 0.1
85D00951	15	8	7	53	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.1	3.7 ± 0.1
85D00952	14	6	8	43	3.3 ± 0.3	2.9 ± 0.3	3.7 ± 0.1	3.5 ± 0.1
85D00971	15	9	6	60	2.9 ± 0.2	2.5 ± 0.2	3.4 ± 0.1	3.2 ± 0.1
85D00981	10	5	5	50	3.6 ± 0.6	3.4 ± 0.5	3.8 ± 0.3	3.7 ± 0.2
85D00982	11	4	7	36	2.5 ± 0.1	2.4 ± 0.2	3.2 ± 0.1	3.1 ± 0.1
85D00995	12	6	6	50	2.5 ± 0.2	2.3 ± 0.3	3.2 ± 0.1	3.0 ± 0.2
86D00002	16	6	10	38	2.7 ± 0.1	2.6 ± 0.1	3.3 ± 0.1	3.2 ± 0.1
86D00003	16	13	3	81	3.3 ± 0.3	3.0 ± 0.2	3.4 ± 0.2	3.4 ± 0.1
86D00004	14	6	8	43	3.0 ± 0.4	2.8 ± 0.1	3.4 ± 0.2	3.3 ± 0.1
86D00015	7	3	4	43	3.0 ± 0.2	3.2 ± 0.1	3.4 ± 0.2	3.5 ± 0.0
86D00034	14	7	7	50	3.0 ± 0.2	2.7 ± 0.2	3.4 ± 0.1	3.3 ± 0.2
86D00035	12	8	4	67	3.6 ± 0.1	3.2 ± 0.1	3.7 ± 0.1	3.6 ± 0.1
86D00036	11	7	4	64	2.8 ± 0.1	2.5 ± 0.1	3.4 ± 0.2	3.4 ± 0.2
86D00041	3	2	1	67	3.5 ± 0.2	3.1	3.6 ± 0.1	3.5
86D00048	14	8	6	57	2.9 ± 0.1	2.7 ± 0.1	3.3 ± 0.1	3.2 ± 0.2
86D00075	7	3	4	43	3.6 ± 0.5	3.6 ± 0.1	3.5 ± 0.2	3.5 ± 0.1

Fetal External Examination - Control Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00001	D	Hematoma on hindpaw	Anasarca; Abnormal body shape (short, thick)

Fetal External Examination - 100 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00051	C	Hematoma on tip of tail	

Fetal External Examination - 316 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00968	M		Bilateral anophthalmia; Hypoplastic pinnae; Lower jaw absent; Abnormal body shape (square)
86D00968	M		Anasarca, severe through thorax

Fetal External Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00034	H	Lips scalloped at edges	
86D00048	B	Hematoma on cranium	

Fetal Visceral Examination - Control Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00955	D	Dilated renal pelvis	
	F	Dilated renal pelvis	
	J	Dilated renal pelvis	
85D00959	D	Dilated renal pelvis	
86000001	D	Dilated renal pelvis	Enlarged adrenals
86D00016	F	Dilated renal pelvis	
	J	Dilated renal pelvis	
86D00071	D	Dilated renal pelvis; Dilated 4th brain ventricle	
	F	Dilated 4th brain ventricle	
	H	Dilated renal pelvis	
	J	Dilated renal pelvis	
	L	Dilated renal pelvis; Dilated 4th brain ventricle	

Fetal Visceral Examination - 100 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00947	B	Dilated renal pelvis	
85D00972	D	Dilated 4th brain ventricle	
86D00024	G	Dilated renal pelvis	
86D00043	B	Dilated renal pelvis	
	H	Dilated renal pelvis	
	J	Dilated renal pelvis	

Fetal Visceral Examination - 316 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00917	C	Dilated renal pelvis	
85D00985	I	Coarse textured, discolored lung	
86D00008	J	Dilated renal pelvis	
86D00027	I	Dilated 4th brain ventricle	
86D00064	H		Left lens small (1/4 normal size); left eyeball position medial
86D00068	M	Dilated 4th brain ventricle; Undescended testes	Partial cleft palate; Abnormal heart (ventricle walls thin, porous texture, hypoplastic papillary muscles, cavities enlarged); surface of lungs lobular; hypoplasia of lungs
86D00080	L	Dilated renal pelvis	

Fetal Visceral Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00942	F	Dilated 4th brain ventricle	
	H	Dilated 4th brain ventricle	
85D00948	C	Dilated 4th brain ventricle	
85D00981	B	Dilated renal pelvis; Dilated nasal cavity	
	F	Dilated renal pelvis	
85D00982	I	Mottled coloration of liver	
85D00995	B	Dilated brain lateral ventricles	
	F	Dilated 4th brain ventricle	
86D00003	F	Dilated renal pelvis	
86D00035	J	Dilated 4th brain ventricle	
86D00048	H	Dilated 4th brain ventricle	

Fetal Skeletal Examination - Control Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00944	A	Retarded ossification pubis	
85D00945	M	Retarded ossification T5 centrum	
85D00955	A	Retarded ossification frontal, parietal, interparietal, supraoccipital; Rudimentary lumbar rib; Retarded ossification pubis; Retarded ossification zygomatic arch; Orbit small; Fewer than 3 caudal vertebrae ossified	
	E	Retarded ossification parietal; Rudimentary lumbar rib	
	G	Retarded ossification interparietal	
	I	Rudimentary lumbar rib	
	K	Retarded ossification frontal, parietal, interparietal, supraoccipital; Rudimentary lumbar rib	
	M	Retarded ossification parietal; Rudimentary lumbar rib;	
	Q	Retarded ossification parietal	
85D00958	M	Rudimentary lumbar rib	
85D00974	A	Retarded ossification parietal	
	J	Rudimentary lumbar rib	
	N	Rudimentary lumbar rib	
85D00976	L	Rudimentary lumbar rib	

Fetal Skeletal Examination - Control Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00988	A	Rudimentary lumbar rib	
86D00001	A	Rudimentary lumbar rib	
	C	Rudimentary lumbar rib	
86D00005	A	Fewer than 3 caudal vertebrae ossified	
86D00010	C	Fewer than 3 caudal vertebrae ossified	
	G	Fewer than 3 caudal vertebrae ossified; Fewer than 3 sternebrae ossified; Fewer than 4 metatarsals ossified; Pubis absent (left); Retarded ossification pubis (right)	
	I	Fewer than 3 caudal vertebrae ossified	
86D00016	E	Retarded ossification frontal; Rudimentary lumbar rib	
	G	Rudimentary lumbar rib	
86D00025	A	Rudimentary lumbar rib	
	D	Rudimentary lumbar rib	
	F	Rudimentary lumbar rib	
	H	Rudimentary lumbar rib	
	N	Rudimentary lumbar rib	

Fetal Skeletal Examination - Control Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00052	C	Rudimentary lumbar rib	
86D00055	F	Fewer than 4 metatarsals ossified; Sternebrae split; Fewer than 3 caudal vertebrae ossified;	
	P	Retarded ossification frontal, parietal; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	
86D00077	I	Rudimentary lumbar rib	
	K	Rudimentary lumbar rib	
	M	Rudimentary lumbar rib; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Retarded ossification: Cervical vertebral arches T14 centrum Pubis absent; Fewer than 4 metatarsals ossified	
86D00079	F	Rudimentary lumbar rib	
	H	Rudimentary lumbar rib	
	J	Rudimentary lumbar rib	

Fetal Skeletal Examination - 100 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00947	I	Rudimentary lumbar rib	
85D00953	G	Rudimentary lumbar rib; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified	
85D00956	E	Rudimentary lumbar rib	
	G	Projections from L4 vertebrae	
85D00972	G	Retarded ossification pubis	
	K	Retarded ossification pubis	
85D00991	A	Pubis short	
85D07992	C	Rudimentary lumbar rib	
86D03020	A	Rudimentary lumbar rib	
	H	Pubis absent (left); Retarded ossification pubis (right); Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	

Fetal Skeletal Examination - 100 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00024	A	Rudimentary lumbar rib	
86D00029	C	Rudimentary lumbar rib; Pubis absent; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	
	E	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib; Retarded ossification parietal	
	K	Rudimentary lumbar rib; Retarded ossification pubis	
	M	Rudimentary lumbar rib	
86D00031	G	Rudimentary lumbar rib	
	I	Rudimentary lumbar rib	
	M	Rudimentary lumbar rib	
86D00032	A	Rudimentary lumbar rib	
	E	Rudimentary 2nd thoracic rib	
86D00037	E	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib	
86D00046	A	Fewer than 3 caudal vertebrae ossified	

Fetal Skeletal Examination - 100 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00054	A	Rudimentary lumbar rib	
86D00073	B	Rudimentary lumbar rib	
	J	Rudimentary lumbar rib	
86D00081	G	Rudimentary lumbar rib	

Fetal Skeletal Examination - 316 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00915	A	Projections from L4 vertebral arch	
85D00917	H	Rudimentary lumbar rib	
85D00938	A	Retarded ossification pubis	
	C	Retarded ossification pubis	
85D00954	G	Rudimentary lumbar rib	
	K	Retarded ossification pubis	
85D00968	A	Rudimentary lumbar rib	
	M	Retarded ossification interparietal; Supraoccipital misshaped; Ribs (right 8-13) bunched, not parallel; Sternebrae abnormal shape; Retarded ossification T5 centrum; T9 centrum misshaped; Femur slightly curved; Fewer than 4 metatarsals ossified	Abnormal orbit (small, slit-like; straight zygomatic arch); Malformed mandible (extremely short, fused on midline); 7 lumbar vertebrae; Cleft palate
85D00993	C	Pubis short	
	J	Pubis short	

Fetal Skeletal Examination - 316 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00008	A	Retarded ossification parietal	
	D	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib	
86D00022	A	Rudimentary lumbar rib	
	C	Rudimentary lumbar rib	
	E	Rudimentary lumbar rib	
86D00026	C	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib	
	O	Rudimentary lumbar rib	
86D00027	M	Rudimentary 2nd thoracic rib	
86D00058	E	Rudimentary lumbar rib	
86D00064	C	Rudimentary lumbar rib	
86D00068	A	Retarded ossification pubis	
	G	Pubis absent (left); Retarded ossification pubis (right)	
	I	Rudimentary lumbar rib	
	K	Retarded ossification pubis	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00942	E	Fewer than 3 caudal vertebrae ossified; Pubis absent; Fewer than 4 metatarsals ossified	
	G	Fewer than 3 caudal vertebrae ossified	
	I	Retarded ossification pubis	
	K	Rudimentary lumbar rib; Fewer than 3 caudal vertebrae ossified	
	O	Rudimentary lumbar rib	
85D00949	B	Retarded ossification supraoccipital; Fewer than 3 sternebrae ossified; Sternebrae split; Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis; Fewer than 4 metatarsals ossified	
	D	Retarded ossification pubis; Sternebrae split	
	F	Retarded ossification pubis; Fewer than 4 metatarsals ossified	
85D00951	G	T3 and T4 centra not ossified	
85D00952	A	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib	
85D00981	A	Pubis short	
	J	Rudimentary lumbar rib	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00982	B	Retarded ossification pubis	
	D	Retarded ossification frontal, parietal, interparietal, supraoccipital; Retarded ossification pubis; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Sternebrae split; Retarded ossification, cervical vertebral arches	
	L	Retarded ossification frontal, parietal, interparietal, supraoccipital; Fewer than 3 caudal vertebrae ossified	
	N	Retarded ossification frontal, parietal, interparietal, supraoccipital; Retarded ossification pubis; Sternebrae split	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
85D00995	A	Sternebrae split; Retarded ossification pubis	
	C	Sternebrae split; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis	
	E	Retarded ossification pubis; Sternebrae split; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified;	
	G	Sternebrae split; Retarded ossification pubis	
	J	Retarded ossification T6 centrum	
	M	Retarded ossification frontal, parietal, interparietal, and supraoccipital; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00002	A	Fewer than 3 caudal vertebrae ossified	
	C	Fewer than 3 caudal vertebrae ossified; Pubis absent	
	E	Fewer than 3 caudal vertebrae ossified; Pubis absent; Fewer than 3 sternebrae ossified; Fewer than 4 metatarsals ossified	
	G	Pubis absent; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	
	I	Pubis absent; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified	
	K	Rudimentary lumbar rib; Pubis absent; Fewer than 3 caudal vertebrae ossified	
	M	Retarded ossification pubis; Fewer than 3 caudal vertebrae ossified	
	O	Retarded ossification pubis; Fewer than 4 metatarsals ossified	
86D00003	C	Rudimentary lumbar rib	
	M	Rudimentary lumbar rib	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00015	A	Lumbar ribs; Retarded ossification pubis; Retarded ossification T2 centrum	
	C	Lumbar ribs; Fewer than 3 caudal vertebrae ossified	
	E	Rudimentary lumbar rib	
	G	Rudimentary lumbar rib; Retarded ossification pubis	
86D00034	A	Retarded ossification frontal; Fewer than 3 caudal vertebrae ossified	
	C	Fewer than 3 sternebrae ossified	
	G	Fewer than 3 caudal vertebrae ossified	
	H	Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified	
	M	Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Fewer than 4 metatarsals ossified; Retarded ossification pubis	
86D00035	A	Fewer than 3 caudal vertebrae ossified	
	C	Pubis short	
	I	Pubis short	
	K	Pubis short	
	M	Rudimentary lumbar rib	

Fetal Skeletal Examination - 1000 mg/kg Nitroguanidine Animals

Maternal ID	Fetal ID	Description of Variation	Description of Malformation
86D00036	A	Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis; Fewer than 4 metatarsals ossified	
	D	Retarded ossification pubis; Fewer than 3 caudal vertebrae ossified; Fewer than 3 sternebrae ossified; Fewer than 4 metatarsals ossified; Rudimentary lumbar rib	
	F	Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis; Fewer than 4 metatarsals ossified; Mandible straight	Malformed orbit (frontal bone does not curve inward to form eyesocket)
	H	Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis	
	J	Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified; Retarded ossification pubis; Fewer than 4 metatarsals ossified	
	L	Retarded ossification pubis; Fewer than 3 sternebrae ossified; Fewer than 3 caudal vertebrae ossified	
86D00048	C	Retarded ossification pubis	
	E	Rudimentary lumbar rib	

Mean Fetal Ossification Data - Control Animals

Maternal ID	Number of Fetuses	Sternebrae	Caudal Vertebrae	Metacarpals Per Paw	Metatarsals Per Paw
85D00939	5	5	4	3	4
85D00944	4	5	5	3	4
85D00945	5	5	4	3	4
85D00955	9	5	4	3	4
85D00958	9	4	4	3	4
85D00959	7	6	5	3	4
85D00974	9	5	5	3	4
85D00976	7	6	5	4	4
85D00986	5	4	4	3	4
85D00988	7	6	5	4	4
86D00001	3	5	4	3	4
86D00005	8	4	3	3	4
86D00010	6	3	3	3	4
86D00014	6	4	4	3	4
86D00016	5	4	4	3	4
86D00025	7	6	6	4	4
86D00039	6	4	4	3	4
86D00052	7	5	4	3	4
86D00055	8	4	3	3	4
86D00071	6	4	4	3	4
86D00076	4	5	5	4	4
86D00077	7	5	4	3	4
86D00079	7	5	5	3	4

Mean Fetal Ossification Data - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	Number of Fetuses	Sternebrae	Caudal Vertebrae	Metacarpals Per Paw	Metatarsals Per Paw
85D00947	7	6	5	3	4
85D00950	8	5	4	3	4
85D00953	5	5	4	3	4
85D00956	9	5	4	3	4
85D00972	6	4	4	3	4
85D00991	8	6	5	3	4
85D00992	8	6	5	4	4
86D00020	6	5	5	4	4
86D00024	5	5	4	3	4
86D00029	5	3	3	3	4
86D00031	7	6	6	4	4
36D00032	6	5	5	3	4
86D00037	6	6	6	4	5
86D00043	7	4	4	3	4
86D00046	6	5	4	3	4
86D00051	6	5	4	3	4
86D00054	7	6	5	3	4
86D00073	8	5	4	3	4
86D00081	7	6	5	3	4

Mean Fetal Ossification Data - 316 mg/kg/day Nitroguanidine Animals

Maternal ID	Number of Fetuses	Sternebrae	Caudal Vertebrae	Metacarpals Per Paw	Metatarsals Per Paw
85D00914	8	5	4	3	4
85D00915	6	5	5	3	4
85D00917	8	4	4	3	4
85D00938	7	4	4	3	4
85D00954	6	5	5	3	4
85D00968	8	5	4	3	4
85D00977	6	5	5	4	4
85D00980	6	6	5	3	4
85D00985	7	6	5	3	4
85D00993	7	5	4	3	4
86D00008	6	6	6	4	4
86D00022	6	6	7	4	4
86D00026	8	5	4	3	4
86D00027	7	5	4	3	4
86D00058	3	6	5	3	4
86D00064	6	4	4	3	4
86D00068	6	4	4	3	4
86D00080	6	6	5	3	4

Mean Fetal Ossification Data - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Number of Fetuses	Sternebrae	Caudal Vertebrae	Metacarpals Per Paw	Metatarsals Per Paw
85D00942	8	3	3	3	4
85D00948	3	3	3	3	3
85D00951	7	6	4	3	4
85D00952	7	5	5	3	4
85D00971	8	5	4	3	4
85D00981	5	5	4	3	4
85D00982	6	3	3	3	4
85D00995	6	3	3	3	4
86D00002	8	3	2	3	4
86D00003	8	4	4	3	4
86D00004	7	4	4	3	4
86D00015	4	3	3	3	4
86D00034	8	3	3	3	4
86D00035	6	4	3	3	4
86D00036	6	2	2	3	3
86D00041	2	5	4	3	4
86D00048	7	4	3	3	4
86D00075	4	4	4	3	4

Incidence of Fetal Examination Findings - Control Animals

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %
85D00939	9	0	0	0	0	4	0	0	0	0	5	0	0	0	0
85D00944	8	0	0	0	0	4	0	0	0	0	4	0	0	1	25
85D00945	9	0	0	0	0	4	0	0	0	0	5	0	0	1	20
85D00955	17	0	0	0	0	8	0	0	3	38	9	0	0		78
85D00958	18	0	0	0	0	9	0	0	0	0	9	0	0	1	11
85D00959	13	0	0	0	0	6	0	0	1	17	7	0	0	0	0
85D00974	17	0	0	0	0	8	0	0	0	0	9	0	0	3	33
85D00976	13	0	0	0	0	6	0	0	0	0	7	0	0	1	14
85D00986	10	0	0	0	0	5	0	0	0	0	5	0	0	0	0
85D00988	14	0	0	0	0	7	0	0	0	0	7	0	0	1	14
86D00001	6	1	17	1	17	3	1	33	1	33	3	0	0	2	67
86D00005	15	0	0	0	0	7	0	0	0	0	8	0	0	1	13
86D00010	11	0	0	0	0	5	0	0	0	0	6	0	0	3	50
86D00014	12	0	0	0	0	6	0	0	0	0	6	0	0	0	0
86D00016	9	0	0	0	0	4	0	0	2	50	5	0	0	2	40
86D00025	13	0	0	0	0	6	0	0	0	0	7	0	0	5	71
86D00039	12	0	0	0	0	6	0	0	0	0	6	0	0	0	0
86D00052	14	0	0	0	0	7	0	0	0	0	7	0	0	1	14
86D00055	15	0	0	0	0	7	0	0	0	0	8	0	0	2	25
86D00071	12	0	0	0	0	6	0	0	5	83	6	0	0	0	0
86D00076	7	0	0	0	0	3	0	0	0	0	4	0	0	0	0
86D00077	13	0	0	0	0	6	0	0	0	0	7	0	0	3	43
86D00079	15	0	0	0	0	8	0	0	0	0	7	0	0	3	43

Incidence of Fetal Examination Findings - 100 mg/kg/day Nitroguanidine Animals

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %
85D00947	14	0	0	0	0	7	0	0	1	14	7	0	0	1	14
85D00950	15	0	0	0	0	7	0	0	0	0	8	0	0	0	0
85D00953	10	0	0	0	0	5	0	0	0	0	5	0	0	1	20
85D00956	17	0	0	0	0	8	0	0	0	0	9	0	0	2	22
85D00972	12	0	0	0	0	6	0	0	1	17	6	0	0	2	33
85D00991	16	0	0	0	0	8	0	0	0	0	8	0	0	1	13
85D00992	15	0	0	0	0	7	0	0	0	0	8	0	0	1	13
86D00020	12	0	0	0	0	6	0	0	0	0	6	0	0	2	33
86D00024	10	0	0	0	0	5	0	0	1	20	5	0	0	1	20
86D00029	9	0	0	0	0	4	0	0	0	0	5	0	0	5	100
86D00031	14	0	0	0	0	7	0	0	0	0	7	0	0	3	43
86D00032	12	0	0	0	0	6	0	0	0	0	6	0	0	2	33
86D00037	11	0	0	0	0	5	0	0	0	0	6	0	0	2	33
86D00043	13	0	0	0	0	6	0	0	3	50	7	0	0	0	0
86D00046	12	0	0	0	0	6	0	0	0	0	6	0	0	1	17
86D00051	11	0	0	1	9	5	0	0	0	0	6	0	0	0	0
86D00054	13	0	0	0	0	6	0	0	0	0	7	0	0	1	14
86D00073	15	0	0	0	0	7	0	0	0	0	8	0	0	2	25
86D00081	14	0	0	0	0	7	0	0	0	0	7	0	0	1	14

Incidence of Fetal Examination Findings - 316 mg/kg/day Nitroguanindine Animals

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %
85D00914	15	0	0	0	0	7	0	0	0	0	8	0	0	0	0
85D00915	11	0	0	0	0	5	0	0	0	0	6	0	0	1	17
85D00917	15	0	0	0	0	7	0	0	1	14	8	0	0	1	13
85D00938	13	0	0	0	0	6	0	0	0	0	7	0	0	2	29
85D00954	12	0	0	0	0	6	0	0	0	0	6	0	0	2	33
85D00968	15	1	7	0	0	7	0	0	0	0	8	1	13	2	25
85D00977	11	0	0	0	0	5	0	0	0	0	6	0	0	0	0
85D00980	12	0	0	0	0	6	0	0	0	0	6	0	0	0	0
85D00985	14	0	0	0	0	7	0	0	1	14	7	0	0	0	0
85D00993	14	0	0	0	0	7	0	0	0	0	7	0	0	2	29
86D00008	11	0	0	0	0	5	0	0	1	20	6	0	0	3	50
86D00022	11	0	0	0	0	5	0	0	0	0	6	0	0	3	50
86D00026	15	0	0	0	0	7	0	0	0	0	8	0	0	3	38
86D00027	13	0	0	0	0	6	0	0	1	17	7	0	0	1	14
86D00058	6	0	0	0	0	3	0	0	0	0	3	0	0	1	33
86D00064	11	0	0	0	0	5	1	20	0	0	6	0	0	1	17
86D00068	13	1	8	0	0	7	1	14	1	14	6	0	0	4	67
86D00080	12	0	0	0	0	6	0	0	1	17	6	0	0	0	0

Incidence of Fetal Examination Findings - 1000 mg/kg/day Nitroguanidine Animals

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Malformed No.	%	Variants No.	%	Number Examined	Malformed No.	%	Variants No.	%	Number Examined	Malformed No.	%	Variants No.	%
85D00942	15	0	0	0	0	7	0	0	2	29	8	0	0	5	63
85D00948	6	0	0	0	0	3	0	0	1	33	3	0	0	3	100
85D00951	15	0	0	0	0	8	0	0	0	0	7	0	0	1	14
85D00952	14	0	0	0	0	7	0	0	0	0	7	0	0	2	29
85D00971	15	0	0	0	0	7	0	0	0	0	8	0	0	0	0
85D00981	10	0	0	0	0	5	0	0	2	40	5	0	0	2	40
85D00982	11	0	0	0	0	5	0	0	1	20	6	0	0	4	67
85D00995	12	0	0	0	0	6	0	0	2	33	6	0	0	6	100
86D00002	16	0	0	0	0	8	0	0	0	0	8	0	0	8	100
86D00003	16	0	0	0	0	8	0	0	1	13	8	0	0	2	25
86D00004	14	0	0	0	0	7	0	0	0	0	7	0	0	0	0
86D00015	7	0	0	0	0	3	0	0	0	0	4	0	0	4	100
86D00034	14	0	0	1	7	6	0	0	0	0	8	0	0	5	63
86D00035	12	0	0	0	0	6	0	0	1	17	6	0	0	5	83
86D00036	11	0	0	0	0	5	0	0	0	0	6	1	17	6	100
86D00041	3	0	0	0	0	1	0	0	0	0	2	0	0	0	0
86D00048	14	0	0	1	7	7	0	0	1	14	7	0	0	2	29
86D00075	7	0	0	0	0	3	0	0	0	0	4	0	0	0	0

Incidence of Fetal
Malformations and Variations
Control Animals

Maternal ID	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %
85D00939	9	0	0	0	0
85D00944	8	0	0	1	13
85D00945	9	0	0	1	11
85D00955	17	0	0	10	59
85D00958	18	0	0	1	6
85D00959	13	0	0	1	8
85D00974	17	0	0	3	18
85D00976	13	0	0	1	8
85D00986	10	0	0	0	0
85D00988	14	0	0	1	7
86D00001	6	1	17	3	50
86D00005	15	0	0	1	7
86D00010	11	0	0	3	27
86D00014	12	0	0	0	0
86D00016	9	0	0	4	44
86D00025	13	0	0	5	38
86D00039	12	0	0	0	0
86D00052	14	0	0	1	7
86D00055	15	0	0	2	13
86D00071	12	0	0	5	42
86D00076	7	0	0	0	0
86D00077	13	0	0	3	23
85D00079	15	0	0	3	20

Incidence of Fetal
Malformations and Variations
100 mg/kg/day Nitroguanidine Animals

Maternal ID	Number Examined	Malformed		Variants	
		No.	%	No.	%
85D00947	14	0	0	2	14
85D00950	15	0	0	0	0
85D00953	10	0	0	1	10
85D00956	17	0	0	2	12
85D00972	12	0	0	3	25
85D00991	16	0	0	1	6
85D00992	15	0	0	1	7
86D00020	12	0	0	2	17
86D00024	10	0	0	2	20
86D00029	9	0	0	5	56
86D00031	14	0	0	3	21
86D00032	12	0	0	2	17
86D00037	11	0	0	2	18
86D00043	13	0	0	3	23
86D00046	12	0	0	1	8
86D00051	11	0	0	1	9
86D00054	13	0	0	1	8
86D00073	15	0	0	2	13
86D00081	14	0	0	1	7

Incidence of Fetal
Malformations and Variations
316 mg/kg/day Nitroguanidine Animals

Maternal ID	Number Examined	Malformed		Variants	
		No.	%	No.	%
85D00914	15	0	0	0	0
85D00915	11	0	0	1	9
85D00917	15	0	0	2	13
85D00938	13	0	0	2	15
85D00954	12	0	0	2	17
85D00968	15	1	7	2	13
85D00977	11	0	0	0	0
85D00980	12	0	0	0	0
85D00985	14	0	0	1	7
85D00993	14	0	0	2	14
86D00008	11	0	0	4	36
86D00022	11	0	0	3	27
86D00026	15	0	0	3	20
86D00027	13	0	0	2	15
86D00058	6	0	0	1	17
86D00064	11	1	9	1	9
86D00068	13	1	8	5	38
86D00080	12	0	0	1	8

Incidence of Fetal
Malformations and Variations
1000 mg/kg/day Nitroguanidine Animals

Maternal ID	Number Examined	Malformed No.	Malformed %	Variants No.	Variants %
85D00942	15	0	0	7	47
85D00948	6	0	0	4	67
85D00951	15	0	0	1	7
85D00952	14	0	0	2	14
85D00971	15	0	0	0	0
85D00981	10	0	0	4	40
85D00982	11	0	0	5	45
85D00995	12	0	0	8	67
86D00002	16	0	0	8	50
86D00003	16	0	0	3	19
86D00004	14	0	0	0	0
86D00015	7	0	0	4	57
86D00034	14	0	0	5	36
86D00035	12	0	0	6	50
86D00036	11	1	9	6	55
86D00041	3	0	0	0	0
86D00048	14	0	0	4	29
86D00075	7	0	0	0	0

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (27)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
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